EFFECTS OF TEMPERATURE ON THE POPULATION DYNAMICS, BIOTIC INTERACTIONS, AND DIVERSITY OF FRESHWATER PROTISTS

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THESIS SUMMARY

Temperature is a major ecological driver and it can have interacting effects with other ecological variables. Identifying these interactions is crucial for predicting the effect of ongoing climate change. In this work I investigated the interactive effects of temperature and abiotic (habitat size, substratum type) or biotic (species identity, thermal adaptation) variables on population dynamics, competitive ability, biodiversity, and body size, using chiefly ciliate protists as model organisms. I addressed my research questions by means of microcosm experiments, as well as using a geothermal stream network characterized by a naturally occurring temperature gradient.

In *Chapter Two* I investigated the joint effect of temperature and habitat size on the population dynamics of *Paramecium caudatum* and *Colpidium striatum*, both in monoculture and in competition. Globally, the current increases in temperature and habitat loss are two major drivers of ecological change, and this study provides the first experimental evaluation of their joint effect on population dynamics. I performed a microcosm experiment using habitats of two different sizes (petri dishes containing 14 or 80 ml of culture medium) along a gradient of 12 temperatures (8°C to 29°C). Temperature, habitat size, and the presence of a competitor affected the population dynamics of *P. caudatum* and *C. striatum*, also producing species-specific and population-parameter-specific interactive effects. The observed effect of habitat size may be due to edge effects influencing ciliates' grazing, an hypothesis that requires further testing.

In *Chapter Three* I investigated the effect of thermal adaptation on population dynamics and on competitive ability, a topic rarely tackled in controlled experimental conditions. In two experiments, I exposed *P. caudatum* and *C. striatum* to relatively low or high temperatures for 22 months (at least 130 generations) and measured the intrinsic growth rate r, approximate carrying capacity $\hat{K}$, and competitive ability of the resulting cold- and warm-adapted populations at different temperatures. Thermal adaptation affected population dynamic parameters differently in the two species. Thermal adaptation affected the thermal response of r, $\hat{K}$, and competitive ability of *P. caudatum* but not of C. *striatum*. This study shows that thermal adaptation can occur on relatively short time scales, and may allow some species to cope with climate change.

In *Chapter Four* I described the effect of a naturally occurring temperature gradient on the alpha diversity, beta diversity, and total biomass of ciliate assemblages found on two different substrates. I studied ciliate assemblages from 13 geothermally heated streams in Hengill, SW Iceland, with temperatures ranging from 5°C to 20°C. In each stream I sampled sandy bottoms and submerged rock surfaces. I found that on rock surfaces, both total ciliate biomass and the alpha diversity were negatively correlated with temperature, and beta diversity increased with increasing temperature difference (ciliate assemblages from warm streams being composed chiefly of subsets of taxa found in colder streams). On the contrary, the composition and total biomass of ciliates from sandy substrates were independent from temperature. Substrate-specific responses may be due to differences in mechanical disturbance, nutrient availability, or exposure to invertebrate grazers.

In *Chapter Five* I used the same field system as in *Chapter Four* to test the validity and generality of the Temperature–Size Rule (TSR), which posits a negative relationship between body size and temperature, in natural ectotherm assemblages. In an observational study, I measured the temperature–body size relationship of benthic, heterotrophic ciliate protists, shelled amoebae, nematodes, and rotifers, sampling rock surfaces from five streams with temperatures ranging from 5°C to 20°C. Moreover, in a warming experiment, I compared the body size of the same groups of organisms from the unwarmed (~6°C) and warmed (9-10°C) sections of a stream, sampling both sandy substrates and rock surfaces. Body size was generally unrelated to temperature, though Pseudomicrothoracida ciliate protists in the observational study and shelled amoebae from sandy substrates in the warming experiment followed the TSR. I suggest that temperature variability in nature may be sufficient to prevent the general effects of mean temperature on body size. Alternatively, species body size may respond differently to temperature depending on their temperature niche optimum.

To conclude, in my thesis I show that biotic and abiotic factors can alter the ecological effects of temperature. The research presented in my thesis supports the effectiveness of both microcosm experiments and natural experiments for testing ecological theories and evaluating their robustness in the face of the variability and complexity of natural systems.

ZUSAMMENFASSUNG

Die Temperatur ist einer der bedeutendsten ökologischen Einflussfaktoren, mit wechselwirkenden Effekten auf zahlreiche ökologische Variablen. Um die Auswirkungen des anhaltenden Klimawandels vorhersagen zu können, ist es entscheidend diese Wechselwirkungen zu identifizieren und zu verstehen. In der vorliegenden Arbeit untersuchte ich die Auswirkungen von Temperatur und anderen abiotische (Habitatgröße, Substrattyp) und biotischen Faktoren (verschiedene Arten, Anpassung an die Umgebungstemperatur) auf die Dynamik von Populationen, die Konkurrenzfähigkeit von Arten, die Biodiversität und die Körpergröße. Laborexperimente mit Mikrokosmen und Einzellern (Ciliaten) und ein geothermales Flussnetzwerk, welches sich durch einen natürlich auftretenden Temperaturgradienten auszeichnet, dienten mir zur Bearbeitung meiner Forschungsfragen.

In Kapitel 2 untersuchte ich die gemeinsame Wirkungen von Temperatur und Habitatgröße auf die Populationsdynamiken von *Paramecium caudatum* und *Colpidium striatum*, die sowohl in Monokultur als auch unter Konkurrenzbedingungen gehalten wurden. Weltweit steigende Temperaturen und der zunehmende Habitatschwund sind zwei Hauptursachen des globalen Wandels. Unsere Studie liefert als erste Hinweise hinsichtlich ihrer gemeinsamen Wirkung auf die Dynamik von Populationen. Ich führte hierzu ein Mikrokosmos-Experiment mit zwei Habitaten unterschiedlicher Größe (Petrischalen mit 14 bzw. 80 mL Kulturvolumen) entlang eines Temperaturgradienten (12 verschiedenen Stufen zwischen 8 und 29 °C) durch. Temperatur, Habitatgröße und die Gegenwart eines Konkurrenten beeinflussten die Populationsdynamiken von *P. caudatum* und *C. striatum*, und ergaben sowohl Art- als auch Populationsparameter-spezifische wechselwirkende Effekte. Der beobachtete Effekt der Habitatgröße könnte dabei auf Grenzeffekte zurückzuführen sein, welche das Grazingverhalten der Ciliaten beeinflussen. Diese Hypothese bedarf jedoch einer weiteren Überprüfung.

In Kapitel 3 untersuchte ich den Effekt der Anpassung an die Umgebungstemperatur auf die Populationsdynamiken und die Konkurrenzfähigkeit, ein Thema, welches selten unter kontrollierten experimentellen Bedingungen untersucht wird. In zwei Experimenten setzte ich *P. caudatum* und *C. striatum* für 22 Monate (entspricht mindestens 130 Generationen) vergleichsweise niedrigen bzw. hohen Temperaturen aus und maß die intrinsische Wachstumsrate *r*, die approximative

Kapazität $\widehat{K}$ und die Konkurrenzfähigkeit (letztere nur für *P. caudatum*). Diese Studie zeigt, dass die Anpassung an die Umgebungstemperatur auf relative kleinen Zeitskalen stattfinden kann, was es einigen Arten erlauben dürfte dem Klimawandel erfolgreich zu begegnen.

In Kapitel 4 beschreibe ich den Effekt eines natürlich auftretenden Temperaturgradienten auf die α- und β-Diversität, und auf die Gesamtbiomasse der Ciliatengesellschaften, die auf zwei unterschiedlichen Substraten angetroffen wurden. Ich untersuchte Ciliatengesellschaften von 13 geothermisch erwärmten Flüssen in Hengill, im Südwesten Islands. Die Temperaturen reichten hierbei von 5 bis 20 °C. In jedem Fluss beprobte ich sandige Untergründe und überspülte, felsige Oberflächen. Dabei konnte ich feststellen, dass sowohl die Ciliatenbiomasse als auch die α-Diversität negativ mit der Temperatur korrelierten; die β-Diversität nahm mit steigender Temperatur zu (Ciliatengesellschaften aus warmen Flüssen bestanden dabei hauptsächlich aus Teilmengen der Taxa, die in kühleren Flüssen gefunden wurden). Im Gegensatz dazu war die Zusammensetzung und die Gesamtbiomasse der Ciliaten, die auf sandigem Substrat gefunden wurden, unabhängig von der Temperatur. Substrat-spezifische Reaktionen könnten ihren Ursprung in Unterschieden bezüglich meachnischer Störungen, der Verfügbarkeit von Nährstoffen oder dem Ausgesetztsein gegenüber invertebraten Grazern haben.

In Kapitel 5 nutze ich dasselbe System wie in Kapitel 4 um die Validität und Generalisierbarkeit der Temperatur-Größe-Regel zu überprüfen. Diese besagt, dass in Gesellschaften ektothermer (kaltblütiger) Organismen Temperatur und Körpergröße in negativer Beziehung zueinander stehen. In einer Beobachtungsstudie maß ich diesen Zusammenhang für benthische, heterotrophe Ciliaten, Schalenamöben, Nematoden und Rotiferen. Hierzu nahm ich Proben von felsigen Oberflächen in fünf Flüssen, deren Wassertemperaturen zwischen 5 und 20 °C lagen. In einem Erwärmungsexperiment verglich ich die Körpergrößen derselben Organismengruppen die aus regulären (ca. 6 °C) und erwärmten (9-10 °C) Flussabschnitten stammten. Hierfür ich beprobte sowohl die sandigen Substrate als auch die felsigen Oberflächen. Im Allgemeinen stand die Körpergröße in keinerlei Zusammenhang zur Umgebungstemperatur, obgleich einige Ciliaten (*Pseudomicrothoracida*) in der Beobachtungsstudie, sowie Schalenamöben auf sandigem und felsigem Untergrund der Temperatur-Körpergröße-Regel durchaus

folgten. Ich nehme an, dass die natürliche Umweltvariabilität ausreicht um den allgemeinen Auswirkungen der Temperatur-Körpergröße-Regel entgegenzuwirken. Alternativ könnte es auch der Fall sein, dass die Körpergrößen der Arten, abhängig vom Optimum ihrer Temperaturnische, unterschiedlich auf die Umgebungstemperatur reagieren.

Ich gelange in meiner Arbeit zu dem Schluss, dass biotische und abiotische Faktoren im Stande sind die weitverbreiteten ökologischen Auswirkungen der Temperatur zu modulieren. Die in meiner Arbeit präsentierten Forschungsergebnisse untermauern die Effektivität von Mikrokosmosexperimenten und natürlichen Experimenten im Bezug auf die Überprüfbarkeit ökologischer Theorien und der Evaluation ihrer Robustheit angesichts der enormen Variabilität und Komplexität natürlicher Ökosysteme.

(Translated from English by Dr Thomas Massie)

1

General Introduction

GENERAL INTRODUCTION

Temperature has major structuring effects at all levels of biological organization. The rate at which biochemical reactions occur is temperature dependent (Brown et al. 2004). At the cell level, temperature affects both energetic requirements and division rates (Savage et al. 2004). The rate of development, reproduction, and feeding, as well as the behaviour of organisms, are also affected by temperature (Brown et al. 2004). Effects of temperature on individuals scale up to population dynamics (e.g., growth rate, carrying capacity), to the strength of interactions (e.g., competition, predation), and to the functioning of the ecosystems they belong to (e.g., rates of decomposition and of exchange of CO_2 and oxygen) (Brown et al. 2004).

The study of the ecological role of temperature has gained special relevance in the last decades due to ongoing climate change, with a predicted increase of 0.3–4.5°C in mean global temperature by the end of the 21st century (Pachauri and Meyer 2014). The efforts undertaken to build a theoretical framework for understanding, predicting, and quantifying the ecological effects of temperature led to the development of the Metabolic Theory of Ecology (MTE). The major pattern underlying the MTE is the tight correlation between the rates of metabolic reactions and ecological processes. According to this theory, the rate at which ecological processes happen has a positive, exponential correlation with temperature. This exponential correlation reflects the Arrhenius equation that describes the temperature-dependence of metabolic reactions, with a correction to account for differences in body mass among organisms. All ecological processes should display the same degree of dependency to temperature, a reflection of the mean activation evergy of underlying metabolic reactions (Savage et al. 2004; Brown et al. 2004; Allen and Gillooly 2007).

Correlative studies on data with low taxonomic resolution support the predictions of the MTE. Yet, experimental evidence suggests that factors other than body mass can modulate how closely ecological processes follow the Arrhenius equation. For example, the activation energy of metabolic rates can differ among taxa (Meehan 2006), and the dependence of predation rate on temperature can be affected by prey behaviour (Vucic-Pestic et al. 2011). At the ecosystem-level,the positive exponential correlation with temperature is steeper for ecosystem respiration than for

primary production (Yvon-Durocher et al. 2010), likely a reflection of the different degrees of temperature dependence of the metabolism of autotrophic and heterotrophic organisms.

Experimental evaluation of the effects of temperature on population and community dynamics is limited. This is due to the long time scales that such studies require for slow-growing species, as well as to the logistic difficulties presented by manipulating temperature in field experiments (O'Gorman et al. 2014). My overall goal for my thesis was to contribute to filling this gap. I did so by conducting microcosm experiments on populations of ciliate protists, as well as by studying assemblages of ciliate protists and other small invertebrates (amoebae, nematodes, and rotifers) along a naturally occurring temperature gradient.

Study systems

The ecological role of temperature in nature is often studied via correlational studies, in which latitudinal or latitudinal gradients are used as proxies for temperature gradients. Yet, the change in mean temperature along these gradients co-varies with other variables (e.g., seasonal and diel variability, habitat type), which makes it challenging to disentangle their relative effects. Alternatively, warming experiments are performed using greenhouse-like chambers in field experiments (e.g. Arft et al. 1999), but this approach does not allow a fine control on the magnitude of the temperature treatment. Moreover, this approach usually implies only two levels of temperature (natural and warmed), thus not allowing a detailed study of the shape of the relationship between the response variable and temperature. To overcome these limitations I manipulated environmental temperature using incubators. This allowed me to build temperature gradients of up to 12 steps, while keeping temperature variability constant within each incubator. At each temperature I grew populations of ciliate protists according to my experimental treatments, using Petri dishes as standard experimental environments. The results of these microcosm experiments are presented in chapters *Two* and *Three* of this thesis.

Microcosm experiments allow precise testing of explicit hypotheses by fine-scale manipulation of experimental variables (J. A. Drake, Huxel, and Hewitt 1996; J.

M. Drake and Kramer 2012; Fraser and Keddy 1997), and they have been used successfully to study the ecological role of temperature (e.g. Jiang and Kulczycki 2004; Jiang and Morin 2004; Beveridge, Petchey, and Humphries 2010). Yet, microcosms have been criticized for being simplistic and not representative of natural ecosystems (Carpenter 1996). In this regard, the study of geothermally heated ecosystems can provide a convenient compromise between microcosm experiments, field experiments, and correlative studies for the study of the ecological effects of temperature (O'Gorman et al. 2014). Chapters *Four* and *Five* of this thesis are based on studies conduced in a geothermal stream network located in Hengill, South-West Iceland. There, we selected between five (*Chapter Five*) and 13 (*Chapter Four*) streams displaying a gradient of mean water temperatures from 5.4 to 20.2°C. Although there are some minor chemical differences among the streams, these are independent of temperature, which is the major environmental gradient in the system. Moreover, the streams lie within two km from each other, and are therefore biogeographically homogeneous (Adams et al. 2013). These features make the Hengill stream network a 'natural experiment' in which the continuity of the temperature treatment is ensured by nature.

Study organisms

I focused my attention chiefly on freshwater ciliate protists (hereafter 'ciliates'). Ciliates are ectotherms, thus their body temperature is equal to environmental temperature. Therefore, their ecological responses are more closely influenced by environmental temperature than those of endotherms (Jiang & Morin 2004). Moreover, ciliates are small and have short generation times, which makes them ideal model organisms for the study of population and competition dynamics.

In my microcosm experiments I used the free-swimming bacterivore ciliates *Paramecium caudatum* Ehrhard and *Colpidium striatum* Stokes, provided with the bacteria *Serratia marcescens* and *Bacillus cereus* as food. These species have previously been used in microcosm experiments evaluating the ecological effect of temperature (e.g. Jiang and Morin 2004; Palamara et al. 2014; Petchey 2000), providing me with useful background information both on the ecology of the species and on the methodologies to be used.

In the studies based on the Hengill stream network, I looked at the natural ciliate assemblages in their entirety. Also, in *Chapter Five*, I compared their temperature–body size response to that of amoebae, nematodes, and rotifers.

Outline of the thesis

All the chapters of my thesis are the result of collaborative efforts, and they are organized as self-contained articles that have been or will be submitted to peer-reviewed journals.

Chapter Two aims at detecting possible interactive effects between temperature and habitat size on the population dynamics of *P. caudatum* and *C. striatum*, both in monoculture and together. Current increases in temperature and habitat loss are two major drivers of ecological change at the global level, yet the assessment of their joint effects has received limited attention (Mantyka-pringle, Martin, and Rhodes 2012). Our study provides the first experimental evaluation of the joint effect of temperature and habitat size on population dynamics, as well as an experimental test of the predictions of the MTE relatively to intrinsic population growth rates.

Chapter Three evaluates whether thermal adaptation affects the population dynamics and competitive ability of *P. caudatum* and *C. striatum*. Climate change is predicted to cause extinctions (Deutsch et al. 2008), yet these predictions do not account for the ability of organisms to adapt to temperature changes. Our study is one of the few to measure the demographic effects of thermal adaptation in controlled experimental conditions.

Chapter Four describes the correlation of temperature with the diversity and total biomass of natural ciliate communities occurring on different substrata, namely sandy substrata and submerged rock surfaces. Temperature is known to affect the dynamics of ciliate communities (e.g. Norf and Weitere 2010), and the nature of the bottom substratum is also known to affect the abundance and species composition of ciliate assemblages (Gucker & Fischer 2003). Our study tested for interactions between temperature and the substratum type, namely whether ciliate assemblages found on dissimilar substrata respond differently to a temperature gradient.

In *Chapter Five* we measured the correlation between temperature and the size of ciliate cells along the natural temperature gradient of five streams in Hengill, as well as in a warming experiment. We also compared the temperature-size correlation of ciliates with that of amoebae, nematodes, and rotifers. Ectotherm body size exhibits a negative correlation with temperature in the majority of laboratory-based studies (Atkinson 1994), yet the general validity of this pattern in natural systems is disputed (Adams et al. 2013; Rypel 2014). Our study aimed at contributing to testing the generality of the negative temperature–size correlation among ectotherms in natural environments.

In *Chapter Six* I synthetize the results from the previous chapters, I discuss some of the questions left unanswered, and I suggest possible ways to tackle them in future research.

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2

Interactive effects of temperature and habitat size on population dynamics in experimental microbial communities

(Manuscript in preparation)

Interactive effects of temperature and habitat size on population dynamics in experimental microbial communities

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Running headline: Temperature, habitat size, and population dynamics

ABSTRACT

Temperature is a well-known driver of population dynamics and can affect the strength of biotic interactions. Population and community dynamics may also be affected by habitat size, yet there is little information about the joint effect of temperature and habitat size on population dynamics. Understanding the joint ecological effects of temperature and habitat size is crucial for biological conservation and management in the light of ongoing global changes in climate and in habitat availability. We performed a factorial microbial microcosm experiment, measuring the effect of temperature (8 to 29°C in 12 steps) and habitat size (small and large) on the population dynamics of two bacterivore ciliate species (*Paramecium caudatum* and *Colpidium striatum*) grown in monoculture and together. We measured population intrinsic growth rate (r), maximum cell density, and the rate of population decline after maximum density was reached (λ). In both species, r depended only on temperature and in a non-linear fashion, partially supporting metabolism-based predictions. The shape of the dependence of maximum cell density from temperature was species-specific but did not depend on the size of the habitat nor on the presence of interspecific competition. The positive relationship between *P. caudatum*'s λ and temperature was unaffected by habitat size, while it was steepened by interspecific competition. Temperature, habitat size, and interspecific competition exerted a three-way interactive effect on *C. striatum*'s λ which was affected by temperature, positively, only in small habitats and in presence of *P. caudatum*. We show that the responses of population size and decline to temperature and habitat size are species-specific and community-mediated. The response of population dynamics to temperature is affected by the presence of competitors more than by the size of the habitat, although complex interactions can arise. We suggest that previously detected interactions between temperature and habitat availability may be due not only to habitat loss and/or fragmentation, more also to habitat size *per se*.

Keywords: biotic interactions; ciliate protists; *Colpidium*; competition; metabolic theory; microcosm experiment; *Paramecium*.

INTRODUCTION

The ecological key role of temperature has been documented at the level of population, species, community, and ecosystem by many observational and experimental studies (e.g. Walther *et al*. 2002; Parmesan 2006; Kordas *et al*. 2011). Habitat availability, too, has crucial effects on population and community viability, and habitat loss negatively affects biodiversity and the size of populations (Fahrig 2003). Although climate change and habitat loss are two major and co-occurring anthropogenic drivers of change for populations and ecosystems, the possibility of a combined effect of temperature and habitat size on population dynamics and species interactions has been largely overlooked (Mantyka-Pringle *et al*. 2012).

The widespread ecological effects of temperature are largely driven by a single process, namely the exponential increase in metabolic rates with increasing temperature (Brown *et al*. 2004). The Metabolic Theory of Ecology (MTE) predicts that, once corrected for body mass, the parameters of population dynamics and the strength of biotic interactions should scale with temperature in a similar fashion as metabolic rates do (Brown *et al*. 2004). The MTE predicts the intrinsic growth rate of populations to increase with temperature, while their carrying capacity should decrease, and inferior competitors be driven to extinction faster (Brown *et al*. 2004). While correlative evidence across many taxa supports the predictions of MTE (Brown *et al*. 2004; Burnside *et al*. 2014), studies focusing on fewer species or individual taxa provide contrasting results (e.g. Montagnes *et al*. 2003), and only a limited number of studies have tested its predictions experimentally (Price *et al*. 2012).

The ecological effects of habitat size have received close attention, too. Small habitats generally have lower biodiversity, supporting less species and less complex trophic webs than large habitats do (Spencer and Warren 1996a, b; Fahrig 2003). This may be due to the fact that small habitat patches can support fewer individuals than larger patches can, and small populations are more prone to stochastic extinction than larger ones (Pimm 1991). Moreover, species have specific minimum patch size requirements, so they cannot persist in habitat patches smaller than a certain threshold size (Fahrig 2003). In contrast to our understanding of the effect of patch size on biodiversity and on populations, our knowledge of the effect of patch size on the

strength of biotic interactions is limited, and restricted to predator-prey systems. Large habitats can increase the prey time to extinction (Luckinbill 1974); on the other hand, edge effects can lead to unexpected, non-additive increases in the predation intensity in large habitats compared to small ones (Bergström and Englund 2004). To our knowledge, the effect of habitat size on biotic interactions other than predation, such as competition for resources, has yet to be experimentally addressed.

To date, few attempts have been made to evaluate the joint ecological effect of temperature and habitat availability. Mantyka-Pringle *et al*. (2012) extracted data on the effect of habitat loss on biodiversity and species abundance from 168 studies, collected climatic information for each of the study locations, and used this information to test for interactive effects between habitat loss, rate of climate change, and current climate on biodiversity and species abundance. They found that habitat loss has a consistently negative impact on species diversity and on the abundance of individuals per species, and this negative effect is more intense in hot climates. These findings suggest that current estimates of the ecological effects of climate change and habitat loss may be misleading, because they are based on the effects of the individual processes alone while ignoring their possible interactions.

Yet, the study by Mantyka-Pringle *et al*. (2012) left open questions. What are the mechanisms behind the interactive effect of temperature and habitat size on biodiversity and on the population size of individual species? Do the interactive negative effect of temperature and habitat loss act on the dynamics of each population individually, or is the effect mediated by interspecific interactions? Moreover, Mantyka-Pringle *et al*. (2012) did not distinguish between the effects of habitat size *per se* from those of habitat loss and habitat fragmentation. These are aspects of habitat availability that are often difficult to discern and to treat independently, although they affect populations and communities in different ways and at different spatial scales (Fahrig 2003).

Here we present a microcosm-based experimental study devised to address some of these questions. Our study aimed at quantifying the effects of temperature and habitat size *per se*, as well as their interaction on species dynamics in monoculture and in competition, by manipulating two species of ciliates grown alone and together. We measured population intrinsic growth rate (r), maximum cell density, and the rate of

population decline after maximum density was reached (λ) in order to ask: (1) how do the parameters of single population dynamics change with temperature and habitat size? (2) Are observed changes in the population intrinsic growth rate well described by the MTE model? (3) Does the presence of a competitor modify the correlation of maximum cell density to changes in temperature and habitat size?

We expected *r* to increase exponentially with temperature up to an optimal temperature (Brown *et al*. 2004). Species may differ in their optimal temperature, but the response of their *r* to suboptimal temperature should present the same exponential increase with a predicted exponent of 0.6–0.7 (Brown *et al*. 2004). Population intrinsic growth rates are density-independent (Verhulst 1838) and measured in a phase in which resources are not limiting, so we expect *r* to be unaffected by the size of the habitat or the presence of a competing species.

We expected maximum cell density to decrease with temperature, because interspecific competition should increase with increasing temperature (Brown *et al*. 2004). The number of individuals present in a habitat at carrying capacity should increase linearly with habitat size, hence we expected the maximum cell density to be the same in habitats of different sizes. We did not expect interactive effects of temperature and habitat size *per se*, because the size of the available habitat should not affect the metabolism-driven responses of individuals to temperature. On the other hand, we expected interspecific competition to reduce maximum cell density, and this reduction to be larger at high temperature than at low, because of greater interspecific competition for resources (Brown *et al*. 2004).

For the same rationale, we expected to see a positive effect of temperature on the rate of population decline λ. We did not expect habitat size to affect λ, while we expected the presence of interspecific competition to increase λ and to steepen its correlation with temperature.

MATERIALS AND METHODS

The organisms used in the experiment are the free-swimming bacterivore ciliates *Paramecium caudatum* Ehrhard (hereafter *Paramecium*) and *Colpidium striatum* Stokes (hereafter *Colpidium*), obtained from the Carolina Biological Supply Company (CBS). Before the experiment, both species were maintained in separate stock cultures at 18.5°C, provided with bacteria *Serratia marcescens* and *Bacillus cereus* (also from CBS).

In our experiment we grew *Paramecium* and *Colpidium* in monoculture and both together, in habitats of different sizes, and at different temperatures. We manipulated habitat size by using Petri dishes of two different sizes, chosen to resemble as closely as possible the ones used by Spencer and Warren (1996a): small (60x15 mm), with 14 mL of nutrient medium each, and large (140x20 mm), with 80 mL of nutrient medium each. We chose the nutrient volumes so that habitats of both sizes would have approximately the same depth (5 mm) in an attempt to even the rates of gas exchange between air and medium. However, our choices did result in different ratios between microcosm volume, wall area, and upper surface area in habitats of different sizes (Table S1).

We manipulated temperature by establishing a gradient of 12 constant temperatures (8, 11, 14, 15.5, 17, 18.5, 20, 21.5, 23, 24.5, 26, and 29°C), in 12 independent incubators. Incubators were dark during the whole experiment to minimize the chance of contamination by algae. For all combinations of temperature and habitat size, three different species assemblages were set up: *Paramecium* alone; *Colpidium* alone; *Paramecium* and *Colpidium* together. We chose to not replicate within incubators (as this would be pseudoreplication) and to have each incubator at a different temperature as this is appropriate for analysis of temperature effects by regression, and gives greater ability to detect non-linear responses to temperature (Cottingham *et al*. 2005).

The nutrient medium consisted of 0.2 g of dissolved CBS protozoan pellets per 1 L of Chalkley's saline solution (Chalkley 1930). The autoclaved medium was inoculated with *S. marcescens* and *B. cereus* on day -3 of the experiment and kept at room temperature. On day 0 of the experiment, the medium was poured into the experimental units and inoculated with the ciliates, each species at an estimated density

of seven individuals per ml. Ciliate population densities were sampled twice a week over the first 45 days of the experiment, and once a week until the end of the experiment, on day 66. Before each sampling, the volume of medium lost by evaporation was replaced with sterile, distilled water to maintain the concentration of dissolved salts. Sampling consisted of withdrawing a small, known amount of medium from each microcosm and counting the number of individuals of each ciliate species in it under a dissecting microscope. After sampling, the withdrawn volume was returned to its experimental unit to minimize alterations of medium volume and species densities.

To be able to parameterize and test the MTE model while accounting for effects of temperature on cell size (see "Analyses"), we estimated the mean cell mass of *Paramecium* and *Colpidium* at each experimental temperature. At the end of the experiment, the length and width of 15 individuals from *Paramecium* and *Colpidium* monocultures in small habitats at each of the 12 temperatures was measured by means of a Nikon DS-Fi1 camera and the picture analysis software NIS-Elements D version 3.2. At 29°C only nine *Paramecium* and no *Colpidium* cells were found, and no *Paramecium* cells were found at 8°C. Cell volumes (in cubic microns) were then estimated by approximation to a regular ellipsoid:

(Eqn. 1) $$Volume = \pi \cdot Width^2 \cdot \frac{Length}{6}$$

The cell mass was estimated by assuming their density to be 1 g · cm^{-3} (i.e. 1 picogram · μm^{-3}), the same as distilled water, as water is the major component of eukaryotic cells (Kageyama *et al*. 1989). We found that the mean cell mass across the temperature gradient differs for the two species, being 19.2±0.84 ng (mean±SE) for *Colpidium* and 64.4±2.74 ng for *Paramecium*. Cell mass of both species changed non-linearly with temperature (Appendix S1).

Analyses

All statistical analyses were performed using R version 3.0.3 (R Core Team 2014).

Single species dynamics. The ciliate monocultures often decreased in abundance after reaching a plateau because we did not replenish resources during the

experiment (Fig. 1). Thus, we divided the time series into two parts, before and after the maximum population density was reached, and analyzed them separately. The changes in cell density up to the plateau were adequately described by a logistic growth model, which we used to estimate the intrinsic growth rate *r* and the maximum cell density (cells·ml^{-1}) of *Paramecium* and *Colpidium* populations under all the experimental combinations of temperature and habitat size. We performed this with general maximum likelihood methods, using function mle2 from the R package "bbmle" version: 1.0.17 (Bolker *et al*. 2014). The population dynamics after the plateau were used to measure the exponential rate of population decline λ by fitting an exponential function on the untransformed data (Fig. 1). Our approach closely resembles the one used by Clements *et al*. (2014) for modelling population dynamics data of a similar nature.

We evaluated the effects of temperature, habitat size, and their interaction on the changes in the estimates of *r* and maximum cell density using analysis of covariance (ANCOVA). Linear and quadratic effects of temperature were modeled.

In order to test whether the MTE model could adequately describe the patterns observed for *r* across the temperature gradient, we first identified the temperature range at which *r* showed a significant, positive, monotonic correlation with temperature. We did it by means of a segmented regression using package *segmented* (Muggeo 2003). We then tested whether, within this range, *r* changes accordingly to the MTE model:

(Eqn. 2) $$r = i_0 \cdot M^{3/4} \cdot e^{-E/kT}$$

Or accordingly to the Arrhenius equation:

(Eqn. 3) $$r = j_0 \cdot e^{-E/kT}$$

Where:

i_0, j_0 = normalization constants,

M = body mass,

E = activation energy for metabolic reactions,

k = Boltzmann's constant,

T = absolute temperature in K degrees.

Eqn. 2–3 were fitted to the untransformed data using function nls in R. The goodness-of-fit of Eqn. 2 and 3 were compared in each case with a linear regression model, respectively corrected and not corrected for body mass. Furthermore, we evaluated the consistency of activation energy estimates obtained from our data with the value predicted by the MTE (0.6–0.7 eV; Brown *et al*. 2004).

Interspecific competition dynamics. We estimated the effect of interspecific competition in modulating the response of r, maximum cell density, and λ to different temperatures and habitat sizes. This comparison was made by a linear model with either r, maximum cell density, or λ as the response variable and presence or absence of a competitor as a binary explanatory variable. Effects of temperature and habitat size (large or small) on competition were tested for by interaction terms of these explanatory variables with the "presence/absence of competitor" explanatory variable. E.g., a significant interaction between presence/absence of competitor and temperature would indicate that temperature affects the strength of competition. To check for non-linearity, we specified a second-order polynomial correlation between temperature and the population dynamics parameters.

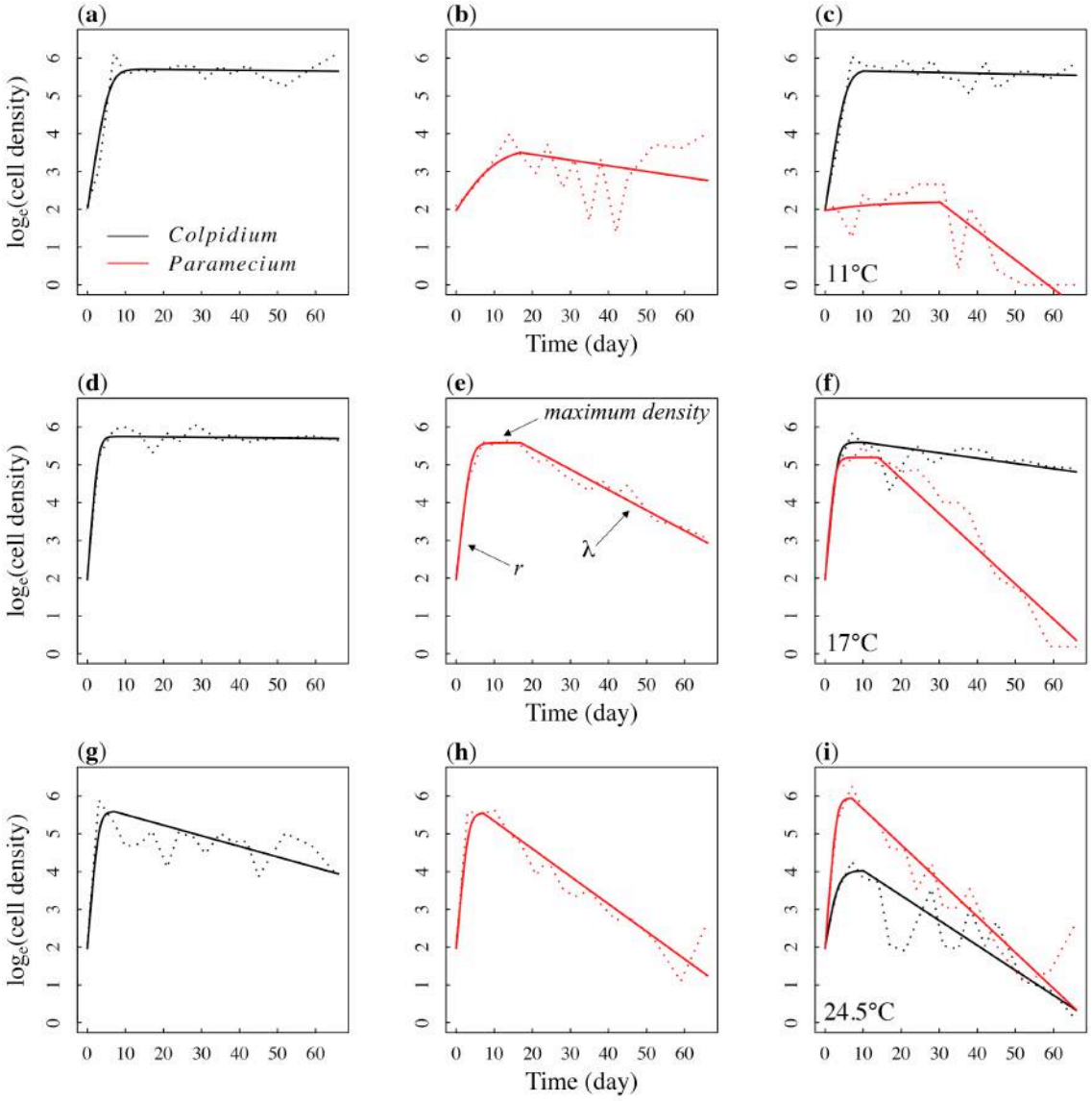

Figure 1. Examples of the observed population dynamics and the models fitted to the data. Data shown refer to the population and competition dynamics of *Colpidium* and *Paramecium* in small habitats at 11 (a-c), 17 (d-f), and 24.5°C (g-i). The population intrinsic growth rate *r* corresponds to the slope at the initial part of the growth curve. The maximum cell density is measured at the plateau after the exponential growth stage. The rate of population decline λ measures the slope of the decline after the maximum cell density is reached (see annotations on panel e).

RESULTS

Temperature affected the population dynamics of both *Paramecium* and *Colpidium* in a species-specific fashion (Fig. 2).

The growth rate *r* was significantly affected by temperature in both *Paramecium* ($F = 88.77$, $df = 2$, $p < 0.0001$) and *Colpidium* ($F = 68.76$, $df = 2$, $p < 0.0001$). *Paramecium*'s *r* increased monotonically with temperature; *r* values reached a plateau at 25°–30°C (Fig. 2a), suggesting that the optimal temperature for this species may be within this temperature range. *Colpidium*'s *r* increased up to ~17°C, then decreased gradually, showing a symmetric, unimodal relationship with temperature (Fig. 2d). Habitat size did not affect *r* of either species, consistently with our expectations. Conversely, competition had an unexpected effect on *Colpidium*'s *r,* which was significantly reduced by the presence of interspecific competition. Moreover, the negative effect of competition on *Colpidium*'s *r* increased with temperature (Tab. S3).

In the temperature ranges in which *r* showed a monotonic positive correlation with temperature, MTE's metabolism-based exponential model (Eqn. 2) performed as well as, or better than a linear model in describing the relationship of *r* and temperature for both species (Table 1). The estimates of activation energy E_a obtained from fitting Eqn. 2 and 3 varied from 0.22±0.08 eV to 0.43±0.11 eV and from 0.41±0.13 eV to 0.76±0.24 eV (Table 1), respectively. Only E_a estimates obtained from the Arrhenius equation for *Paramecium* in large habitats and *Colpidium* in small habitats fell within the mean range of 0.6–0.7 eV reported by Brown *et al*. (2004) (Fig. 3).

The maximum cell density of *Paramecium* displayed a quadratic correlation with temperature ($F = 32.19$, $df = 2$, $p < 0.0001$), generally positive in our experimental temperature range. Conversely, it was not affected by habitat size or interspecific competition (Table S4), although graphical inspection suggested otherwise (Fig. 2b). The maximum cell density of *Colpidium* reached its highest values at temperatures < ca. 17°C, while beyond this threshold it declined with increasing temperature ($F = 102.68$, $df = 2$, $p < 0.0001$)(Fig. 2e). The presence of a competing ciliate species reduced the maximum cell density of *Colpidium*, as expected. It also reduced the positive correlation of *Colpidium*'s maximum cell density with temperature at the low end of our experimental temperature range. The size of the habitat had an unexpected, positive

effect on the maximum cell density of *Colpidium* ($F = 13.25$, $df = 1$, $p < 0.0001$)(Fig. 2e), such that it was approximately 1.25 times larger in large habitats than in small habitats (mean ± SE are 328.36 ± 22.14 and 262.82 ± 22.75, respectively). In other words, the maximum cell density reached by *Colpidium* in small habitats was only ~80% of that reached in large habitats.

The two species differed in the response of λ to the experimental factors (Fig. 2c, f). *Paramecium*'s λ showed a positive, linear correlation with temperature, so that *Paramecium* monocultures declined 0.007 times faster for each one-degree increase in temperature ($SE = 0.001$, $t = 7.901$, $p < 0.0001$)(Fig. 2c). *Paramecium*'s λ was overall higher in small habitats, but its correlation with temperature did not differ in habitats of different sizes; on the other hand, the presence of a competing species reduced the slope of its correlation with temperature (slope = 0.0021, $SE = 0.001$, $t = -3.791$, $p = 0.0005$).

Colpidium's λ showed strikingly different values for temperatures higher and lower than 23°C, so its interpretation required analyzing values from the two temperature ranges separately (Fig. 2f). For temperatures lower than 23°C, a significant interaction between temperature, habitat size and interspecific competition existed; λ of *Colpidium* kept in small habitats with a competing species increased with temperature with slope 0.003 ($SE = 0.0004$, $t = 7.34$, $p < 0.0001$), while *Colpidium*'s λ did not differ from 0 (i.e. populations do not decline) and was unaffected by temperature in all other experimental treatments.

At temperatures higher than 23°C, values of *Colpidium*'s λ in small habitats were coherent with the trend observed at lower temperatures. Conversely, rates of decline in large habitats at temperatures above 23°C were strikingly higher than those observed at lower temperatures (0.571 at 24.5°C and 0.401 at 26°C as opposed to 0.003±0.001 at temperatures < 23°C). The few data points available for temperatures higher than 23°C suggest that, at high temperatures, the presence of *Paramecium* stabilized the dynamics of *Colpidium* populations in large habitats; yet the data points are too few to be meaningfully analysed.

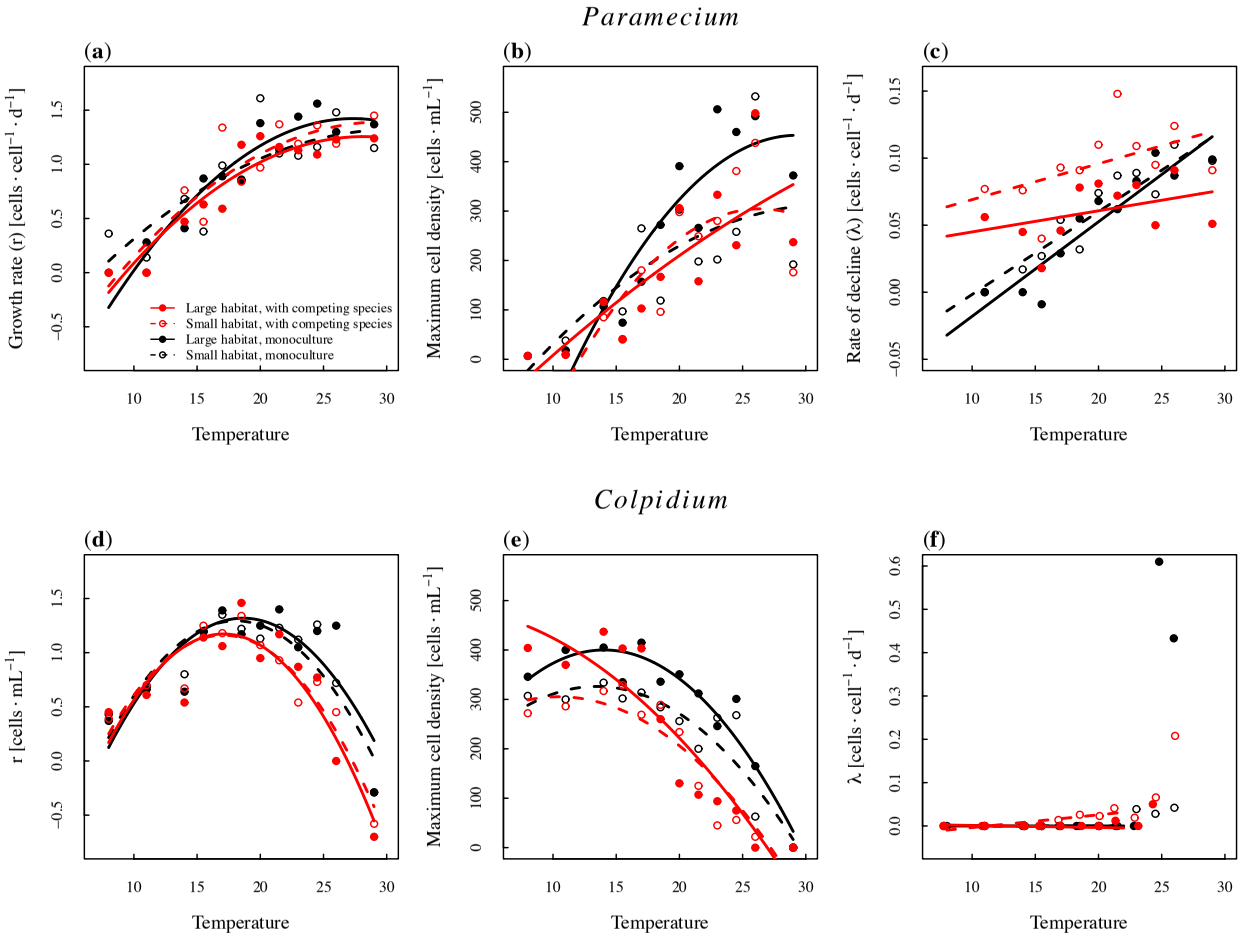

Figure 2. Population dynamics parameters for *Paramecium* (a, b, c) and *Colpidium* (d, e, f) in habitat of two different sizes and with competing species present or absent.

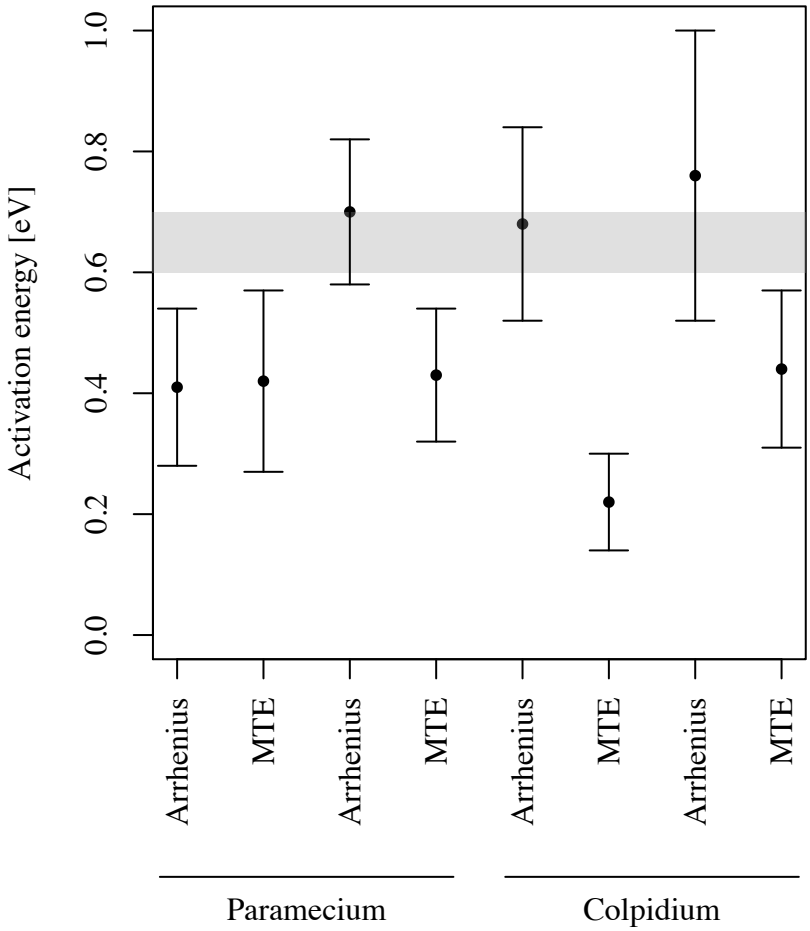

Figure 3. Values of activation energy estimated by fitting either Eqn. 2 (the full MTE equation) or Eqn. 3 (the Arrhenius equation, similar to Eqn. 2 but without taking body size into account) on the values of *r* measured at different temperatures. The fit was performed within the temperature range where *r* showed a monotonic positive correlation with temperature. The grey area delimits the 0.6–0.7 eV range reported to be the average value for activation energy E_a (Brown *et al*. 2004).

Table 1. Comparison of models describing the correlation between the intrinsic growth rate *r* of *Paramecium* and *Colpidium* and temperature. The Arrhenius model and the MTE model are compared to a non-mass-corrected and a mass-corrected linear model (identified by 'm.c.' in the table), respectively. The comparison was performed within the temperature ranges where *r* showed a monotonic positive correlation with temperature.

Species	Habitat size	Model	AIC	Temperature dependence parameter (SE)	t	p
Paramecium	Small	Arrhenius	9.99	$E_a = 0.41$ (0.13)	3.10	0.0119 *
		Linear	7.80	Slope = 0.06 (0.01)	4.17	0.0019 **
		MTE	- 263.14	$E_a = 0.42$ (0.15)	2.68	0.0250 *
		Linear, m.c.	- 264.90	Slope = $2.46 \cdot 10^{-7}$ ($7 \cdot 10^{-8}$)	3.53	0.0064 **
	Large	Arrhenius	-2.07	$E_a = 0.70$ (0.12)	5.74	0.0007 ***
		Linear	-5.40	Slope = 0.01 (0.01)	8.22	< 0.0001 ***
		MTE	-269.30	$E_a = 0.43$ (0.11)	3.94	0.0034 **
		Linear, m.c.	-272.04	Slope = $2.64 \cdot 10^{-7}$ ($5 \cdot 10^{-8}$)	5.23	0.0005 ***
Colpidium	Small	Arrhenius	-2.23	$E_a = 0.68$ (0.16)	4.28	0.0128 *
		Linear	-3.61	Slope = 0.09 (0.01)	5.77	0.0045 **
		MTE	-295.74	$E_a = 0.22$ (0.08)	2.65	0.0267 *
		Linear, m.c.	-296.03	Slope = $4.73 \cdot 10^{-8}$ ($1.63 \cdot 10^{-8}$)	2.90	0.0177 *
	Large	Arrhenius	1.85	$E_a = 0.76$ (0.24)	3.13	0.03522 *
		Linear	1.24	Slope = 0.09 (0.02)	4.03	0.0158 *
		MTE	-287.60	$E_a = 0.44$ (0.13)	3.52	0.0066 **
		Linear, m.c.	-286.65	Slope = $8.76 \cdot 10^{-8}$ ($2.50 \cdot 10^{-8}$)	3.51	0.0066 **

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

DISCUSSION

Both temperature and habitat size have long been known to be important factors affecting population dynamics and biotic interactions. A recent meta-analysis by Mantyka-Pringle *et al*. (2012) showed that temperature and habitat availability may interact, producing effects on biodiversity and the abundance of individuals that are larger than we would expect by examining temperature and habitat availability individually. Yet, their definition of "habitat availability" included both habitat size *per se*, habitat loss, and fragmentation, thus leaving some confusion on which aspect of habitat availability is actually interacting with temperature. Our study addresses some of the issues raised by Mantyka-Pringle *et al*. (2012) and it is, to our knowledge, the first experimental evaluation of the joint effect of temperature and habitat size *per se* on population dynamics.

We expected both temperature and habitat size, individually, to have significant effects on population dynamics, while we did not expect to observe interactive effects because the two factors act via different pathways (mainly metabolic and non-metabolic, respectively). Our results confirmed these expectations only partially.

As we expected, the intrinsic growth rate *r* of both *Paramecium* and *Colpidium* populations was significantly affected by temperature and was independent from the size of the habitat. Unexpectedly, *Colpidium*'s *r* was reduced in presence of interspecific competition. Since *r* is defined as density-independent (Verhulst 1838), the presence of a competing species must reduce *Colpidium*'s growth by density-independent pathways. Protist cells can "leak" cytoplasmic matter in the environment (e.g. Maurin *et al*. 1997), and some protist species are known to be toxic (e.g. Turner and Tester, 1997). It is thus possible that *Paramecium* released substances that resulted detrimental to *Colpidium*'s growth regardless of *Paramecium*'s density. This hypothesis requires testing, for example by comparing *Colpidium*'s growth in presence of *Paramecium* with its growth in presence of *Paramecium*'s exudate, but not of live *Paramecium* cells.

In the temperature range in which intrinsic growth rate showed a positive correlation to temperature, a linear regression model often described the correlation as well as an exponential model, and our estimates of the metabolic activation energy E_a were always significantly lower than the value of 0.6–0.7 eV expected by the MTE (Brown *et al*. 2004). Although a linear model and an exponential one seem equally valid in describing our data from a statistical point of view, we believe the exponential model to be conceptually

preferable because it not only described the observed patterns, but also shed light on the mechanism behind them. Future studies should focus on which factors (metabolism-related or not) can produce shifts from the 0.6–0.7 eV exponent expected by MTE. It must be noted that the reliability of our estimates of E_a may be limited by the small amount of data points they are based on; future studies should focus on the temperature range at which the population parameters of interest are known to be positively correlated to temperature, and maximize the replication within it to increase statistical power. Our results provide a good baseline for such studies.

The maximum cell density reached by both ciliate species was significantly affected by temperature in a non-linear fashion, while its response to changes in habitat size was species-specific. In any case, in accordance to our expectations, temperature and habitat size did not produce interactive effects on maximum cell density of either ciliate species. The maximum cell density of *Paramecium* was significantly affected only by temperature, while the maximum cell density of *Colpidium* also showed an unexpected response to habitat size, such that in small habitats was only 80% of what observed in large habitats. The different response of maximum cell density to changes in habitat size in *Paramecium* and *Colpidium* may be due to edge effects. The ratio between volume and surface area and that between volume and the area of submerged surfaces differed in the two experimental habitats (Table S1). This may affect the grazing activity of *Paramecium* and *Colpidium* on bacteria, and the species-specificity of possible edge effects may be explained by the different dietary preferences in the two ciliate species. In our study we used bacteria species *Serratia marcescens* and *Bacillus cereus*. Previous studies showed that *Colpidium striatum* is a specialist feeder with a preference for *S. marcescens* bacteria, while *Paramecium tetraurelia*, a species affine to *P. caudatum* used in our study, also consumes significant amount of *B. cereus* (Jiang and Morin 2004). If edge effects were to increase the relative abundance of *S. marcescens* over *B. cereus*, this would give an advantage to *Colpidium*, but not to *Paramecium* in larger habitats. This is a conjecture that our data do not allow to test, while it finds some support from previous studies. Bergström and Englund (2004) experimentally observed that the attack rate of a predator shrimp on a cladoceran prey is higher in large arenas than in small ones, even if the initial density of predator and prey was the same in the two arenas. This was due to a preference of both predator and prey for aggregating by the edges of the experimental arena, leading to higher encounter rates between predator and prey in the large arenas than in the small ones. Further studies are required to test this hypothesis in our ciliate-bacteria system.

The only significant interactive effect involving temperature and habitat size regarded *Colpidium*'s λ. For temperatures < 23°C, *Colpidium* populations grown in small habitats and exposed to interspecific competition declined at increasing rate with increasing temperature. In the same temperature range, *Colpidium* populations exposed to all other treatment combinations did not show any decline at all. While we expected an interactive effect of temperature and competition on λ of both species, it is difficult to explain why this interaction was observed for *Paramecium* regardless of habitat size while it depended on the size of the habitat in the case of *Colpidium*. Perhaps the same edge effects hypothesized above may have a role, but this hypothesis requires testing, too.

To conclude, our study suggest that temperature and habitat size *per se* do not produce interactive effects on commonly measured population dynamics parameters such as intrinsic growth rate and the density of individuals at equilibrium. Yet, it can affect the temperature-depencence of population decline rates in species-specific, community-mediated ways.

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SUPPORTING INFORMATION

Table S1. Information about the volume, vertical walls area, superficial surface area, and their ratios in habitats of different sizes.

Habitat size	Volume	Walls area	Surface area	Volume/ Walls area	Volume/ Surface area	Walls area/ Surface area
Small	14 ml	3769.5 mm^2	2827 mm^2	2.65 mm	3.54 mm	1.33
Large	80 ml	17594 mm^2	15394 mm^2	4.55 mm	5.20 mm	1.14

Appendix S1. Effects of temperature on the body size of the experimental organisms

In order to verify whether temperature affected the size of *Paramecium* and *Colpidium* cells, at the end of the experiment the length and width of 15 individuals from *Paramecium* and *Colpidium* monocultures was measured by means of microphotography and the picture analysis software ImageJ. Most of the measured individuals were taken from small habitats, large habitats being used only when cell density was too low. Because of lack of individuals, at 29°C only nine *Paramecium* and no *Colpidium* cells were measured. For the same reason, no *Paramecium* cells were measured at 8°C. Cell mass was estimated following the method described in the main text.

We found that the mean cell mass across the temperature gradient differs significantly for the two species, being 19.2 ng (SD=10.9) for *Colpidium* and 64.4 ng (SD=33.8) for *Paramecium*. Yet their cell size distributions strongly overlap at 23°C and 26°C (Fig. S1).

The dependence of *Colpidium*'s cell mass from temperature is non-linear, best described by a 3rd-degree polynomial model (Tab. S2). The dependence of *Paramecium*'s cell mass from temperature is also best described by a 3rd-degree polynomial model when data collected from all temperatures are considered (Tab. S2).

These results are based on small samples taken mainly from populations cultured in small habitats. Further studies taking into account the effects of temperature in conjunction with those of habitat size on the size of organisms might reveal unexpected patterns.

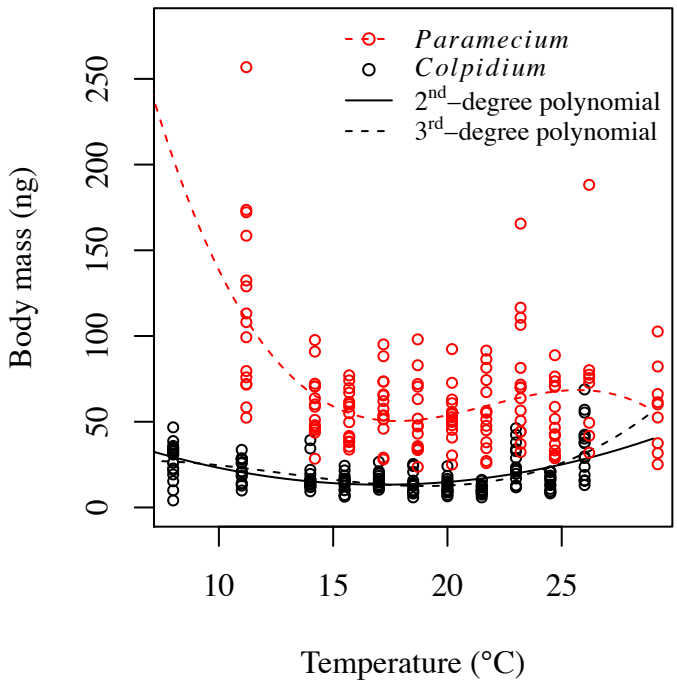

Figure S1. Scatterplots showing the changes in cell size across the temperature gradient for *Colpidium* (black) and *Paramecium* (red). Data for Paramecium are plotted with a +0.2°C shift to facilitate the reading. Refer to Table S2 for the coefficient values and relative significances of the models depicted by the fitted lines.

Table S2. Summary of the different models used to describe the relationship between temperature and cell mass for *Paramecium* and *Colpidium*. The letters indicating the parameters can be interpreted referring to the following general expression: $y = a + b \cdot x + c \cdot x^2 + d \cdot x^3$, where y is cell mass and x is temperature expressed in Celsius degrees.

Species	Model	AIC	Adj. R^2	Parameters	Estimate	SE	t value	P-value	
Colpidium	2nd-degree poly	1225.7	0.26	a	69.7	6.9	10.1	<0.0001	***
				b	-6.6	0.8	-7.7	<0.0001	***
				c	-6.4	0.0	7.8	<0.0001	***
Colpidium	3rd-degree poly	1218.8	0.30	a	9.7	21.2	0.5	0.646	ns
				b	5.9	4.2	1.4	0.167	ns
				c	5.3	0.3	-2.2	0.026	*
				d	5.31	0.0	3	0.003	**
Paramecium	3rd-degree poly	1462.3	0.25	a	740.6	116.0	6.4	<0.0001	***
				b	-100.7	19.0	-5.3	<0.0001	***
				c	-95.9	1.0	4.8	<0.0001	***
				d	-95.97	0.0	-4.4	<0.0001	***

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Table S3. Analysis of Covariance of *r* in dependence of temperature (quadratic), habitat size, and presence of a competing species; ANCOVA selection of the most parsimonious model was performed separately for the two study species.

Species	Source of variation	Df	MS	F	p
Paramecium	Temperature (Tmpt)	2	3.90	88.77	< 0.0001 ***
	Residual	44	0.04		
Colpidium	Tmpt	2	4.57	68.76	< 0.0001 ***
	Cmp	1	0.55	8.28	0.0037 **
	Tmpt · Cmp	2	0.20	3.08	0.0388 *
	Residual	42	0.06		

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Table S4. Analysis of Covariance of maximum cell density in dependence of temperature (quadratic), habitat size, and presence of a competing species; ANCOVA selection of the most parsimonious model was performed separately for the two study species.

Species	Source of variation	Df	MS	F	p
Paramecium	Tmpt	2	305865	32.19	< 0.0001 ***
	Residual	43	9501		
Colpidium	Tmpt	2	311796	102.68	< 0.0001 ***
	Cmp	1	51745	17.04	0.0001 ***
	Hbt	1	28033	9.23	0.0041 **
	Tmpt · Cmp	2	14911	4.91	0.0122 *
	Residual	41	3037		

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Table S5. Analysis of Covariance of λ in dependence of temperature (linear), habitat size, and presence of a competing species; ANCOVA selection of the most parsimonious model was performed separately for the two study species. ([a]) Only temperatures < 23°C were used, because at higher temperatures *Colpidium*'s λ showed strikingly heterogeneous, nonlinear patterns.

Species	Source of variation	Df	MS	F	p
Paramecium	Tmpt	1	0.023	54.51	< 0.0001 ***
	Cmp	1	0.005	12.74	0.0010 **
	Hbt	1	0.005	12.01	0.0013 **
	Tmpt · Cmp	1	0.006	14.37	0.0005 ***
	Residual	39	$4.2 \cdot 10^{-4}$		
Colpidium[a]	Tmpt	1	$4.2 \cdot 10^{-4}$	18.20	0.0003 ***
	Cmp	1	$4.2 \cdot 10^{-4}$	17.96	0.0003 ***
	Hbt	1	$2.6 \cdot 10^{-4}$	11.30	0.0025 **
	Tmpt · Hbt	1	$2.2 \cdot 10^{-4}$	9.44	0.0052 **
	Tmpt · Cmp	1	$4.2 \cdot 10^{-4}$	18.20	0.0003 ***
	Hbt · Cmp	1	$2.6 \cdot 10^{-4}$	11.30	0.0026 **
	Tmpt · Hbt · Cmp	1	$2.2 \cdot 10^{-4}$	9.44	0.0052 **
	Residual	36	$2.3 \cdot 10^{-5}$		

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

3

Effect of thermal adaptation on the population dynamics and competition ability of two ciliate species at different temperatures

(Manuscript in preparation)

Effect of thermal adaptation on the population dynamics and competition ability of two ciliate species at different temperatures

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Running headline: Thermal adaptation and population dynamics

ABSTRACT

Many studies have evaluated the effect of thermal adaptation on individual life history traits but few have addressed its effect on population dynamics and competitive ability. Moreover, most of these studies examined latitudinal or altitudinal gradients, while only a few assessed the effect of long-term exposure to low or high temperatures under controlled experimental conditions. In a microcosm experiment we exposed the ciliate protists *Paramecium caudatum* and *Colpidium striatum* to low and high temperatures (11°C and 24.5°C and 8°C and 20°C, respectively) for 22 months (≥ 130 generations). We then transferred individuals from each of these temperatures to microcosms along a gradient of five temperatures (11, 15.5, 18.5, 20, and 24.5°C, and 8, 11, 15.5, 18.5, and 20°C, respectively), and measured their intrinsic growth rate r and approximate carrying capacity $\hat{K}$. Furthermore, we performed a three-way orthogonal experiment to assess the competitive performance of *P. caudatum* and *C. striatum* pre-exposed to low or high temperatures when kept at low or high temperature in presence of a competing species pre-exposed to low or high temperatures. We found that the temperature dependence of population dynamics of *P. caudatum* depended on temperature-pre-exposure. The temperature dependence of *P. caudatum*'s r was stronger among populations pre-exposed to low temperature compared to that pre-exposed to high temperature. Temperature had a positive effect on *P. caudatum*'s $\hat{K}$, the correlation having the same slope regardless of the temperature of pre-exposure, but with higher cell densities reached in populations pre-exposed to high temperature. The competitive ability of *P. caudatum* changed interactively with the pre-exposure temperature of both *P. caudatum* and *C. striatum*, as well as with the temperature at which the competition trial was conducted. On the contrary, thermal adaptation did not affect the thermal response of *C. striatum*'s r, $\hat{K}$, and competitive ability, although all three parameters were temperature-dependent. Our study shows that some fast-growing ectotherms can evolve to adapt to climate change in ways that have important effects on population and competitive dynamics. Studies that integrate ecology, physiology and genetics are required to understand why and how some species, individual life traits, and ecological parameters are subject to thermal adaptation to different degrees.

Keywords: thermal adaptation; biotic interactions; *Colpidium*; evolution; metabolic theory; microcosm experiment; *Paramecium*.

INTRODUCTION

Understanding the ability of individuals and populations to adapt to different environmental temperatures is crucial to predict the ecological effects of climate change (Deutsch *et al*., 2008; Amarasekare & Savage, 2012). Many studies have measured the effect of adaptation to different environmental temperatures on traits of organisms by sampling along latitudinal or altitudinal gradients (Angilletta, 2009). Yet, few studies have assessed the effect of thermal adaptation on population and community dynamics, and thermal adaptation has rarely been reproduced in controlled environments (Angilletta, 2009). Here we present the results of two laboratory experiments investigating the effect of long-term exposure to constant low and high temperature on the thermal response of population dynamics and competitive ability, using the ciliate protists *Paramecium caudatum* and *Colpidium striatum*.

Environmental temperature affects biological processes at a variety of scales, from individual cells to whole ecosystems (Brown *et al*., 2004). The response of traits of individuals, such as survival, fecundity, locomotion, individual growth, and developmental time to temperature is described by thermal performance curves (TPCs). The shape of TPCs varies among species and populations but is typically unimodal and left-skewed (Ratkowsky *et al*., 2005), reflecting the temperature correlation of underlying biochemical reaction rates (Amarasekare & Savage, 2012). Thermal performance curves are characterised by four parameters. Lower and upper temperature limits, T_{min} and T_{max}, are the lowest and highest temperatures at which the minimum viable values of a biological parameter are recorded, respectively. The optimal temperature, T_{opt}, is the temperature at which a biological variable reaches its maximum, defined as maximum performance (Amarasekare & Savage, 2012). According to the Metabolic Theory of Ecology (MTE), between T_{min} and T_{opt} the correlation of biological variables with temperature is described by the Arrhenius equation, which predicts that biological variables increase exponentially with increasing temperature (Brown *et al*., 2004). The correlation between biological variables and environmental temperature is particularly strong in ectotherms, whose body temperature is more closely influenced by environmental temperature than in endotherms. This makes ectotherms, which encompass > 99% of all living species, particularly prone to the effects of climate change (Deutsch *et al*., 2008; Amarasekare & Savage, 2012).

Thermal performance and adaptation

The expectation of extinctions does not account for the ability of organisms to adapt to temperature changes (Deutsch *et al*., 2008). Theory predicts that, within a species, individuals from populations exposed to different temperatures would evolve TPCs with T_{opt} close to the mean environmental temperature, T_{env}. Populations adapted to different temperatures may reach the same maximum performance if they can adapt towards fully compensating for the thermal limitations of metabolism kinetics ('thermodynamic-constraint' hypothesis) (Clarke, 2003). Alternatively, populations adapted to low temperatures would reach lower maximum performances than populations adapted to high temperatures ('warmer is better' hypothesis) (Heinrich, 1977). The breadth of the TPC is not expected to be affected by changes in mean T_{env}, while it should become broader or narrower if the variability of environmental temperature increases or decreases, respectively (Lynch & Gabriel, 1987; Gilchrist, 1995). Most studies have tested these predictions by using individuals of the same species from populations collected along altitudinal or latitudinal gradients, or by using individuals from closely related species living in different climates and comparing their performances at different temperatures in controlled conditions. Results vary depending on the taxa and on the biological trait measured. For example, the relationships between survival and T_{min} or T_{max} are generally more strongly related to latitude than to T_{opt} or thermal performance breadth (reviewed by Angilletta, 2009). In the case of organisms' developmental rate, heterogeneous responses to local climatic conditions are documented, including both positive and negative correlations between T_{env} and T_{opt} (e.g. Norry *et al*., 2001; Laugen *et al*., 2003; Oufiero & Angilletta, 2006; reviewed by Angilletta, 2009).

Thermal adaptation and population dynamics

Compared to the thermal adaptation of individual traits, the effect of adaptation to natural climatic gradients on population and community dynamics has received considerably less attention. Among the few examples, a comparison of 18 clones of *P. caudatum* collected along a latitudinal gradient in Europe and from three different Indonesian locations revealed that the intrinsic growth rates of tropical clones increase towards higher temperatures, reach higher maximum values, and have narrower TPCs, compared to European clones (Krenek *et al*., 2012). A review of the TPCs for the intrinsic growth rate of 65 insect species from different climatic regions also supported the 'warmer is better' hypothesis, with the maximum

growth rate of each species being best predicted by T_{env} and T_{opt} (Frazier *et al*., 2006). These studies based on naturally occurring altitudinal, latitudinal, or climatic gradients give valuable insights on the mechanisms and direction of thermal adaptation. Yet, the interpretation of their results is complicated by mean and variability of temperature (temperature extremes, seasonality) co-varying along these gradients, making it difficult to disentangle their relative effects on thermal adaptation.

Microcosm and mesocosm experiments can overcome these limitations, but only few studies have used this approach to investigate the effect of thermal adaptation on population and community dynamics. A study on two bacteriophage strains found that, after being exposed to a temperature close to their upper thermal limit over multiple generations, their intrinsic growth rate at that temperature increased (Holder & Bull, 2001). In that study, one strain adapted to high temperatures more quickly than the other, but in both strains the changes in intrinsic growth rate were strongly correlated to the number of accumulated genetic mutations, thus confirming the changes to be evolution-driven. On the contrary, in another study, strains of the bacterium *Escherichia coli* adapted to 32°C, 37°C, or 42°C (close to *E. coli*'s T_{max}) for 2000 generations did not evolve any differences in the TPCs of their intrinsic growth rates (Bennett & Lenski, 1993).

Thermal adaptation can also affect competitive ability. Experimental exposure of the water flea *Daphnia magna* to high temperatures lead to populations that could outcompete cold-adapted conspecifics at both high and low environmental temperatures, and improved their competitive ability against natural populations from warmer climates (Van Doorslaer *et al*., 2009a,b). Similarly, strains of *E. coli* exposed to 32°C, 37°C, and 42°C significantly increased their competitive ability at that temperature, compared to their ancestor (Bennett *et al*., 1990, 1992). Yet, the evolution of competitive ability at 20°C (close to T_{min}) was considerably more limited, suggesting an asymmetry in the evolutionary potential between upper and lower thermal limits (Mongold *et al*., 1996). These findings suggest that there may be trade-offs between the evolutionary ability of biological traits and of TPC parameters, with some species investing more in evolving towards faster growth, or toward being better competitors, and with evolutionary potential at high temperatures being greater than at low temperatures.

Our study

To better understand the adaptation potential of population dynamics and of competitive ability, and their possible trade-offs, we conduced experiments with two ciliate species *Paramecium caudatum* and *Colpidium striatum* from populations exposed long-term (22 months) to different temperatures. *Paramecium striatum* and *C. striatum* have a long history as model organisms in ecological microcosm experiments (e.g. Gause, 1934; Jiang & Morin, 2004; Beveridge *et al*., 2010; Palamara *et al*., 2014), as they are easy to keep, small, and have short generation times. These characteristics make them particularly suitable for addressing ecological and evolutionary questions. To summarise, we studied whether long-term adaptation to different temperatures leads to changes in population dynamics and competition abilities, and the way organisms respond to temperature gradients. To our knowledge, this is the first time that thermal adaptation has been assessed contextually at the levels of intrinsic growth rate, carrying capacity, competitive ability, and the strength of the correlation of these variables with temperature.

Based on theory and on previous observations, the population dynamic parameters *r* and *K* may adapt to temperature in different ways (Bennett & Lenski, 1993; Frazier *et al*., 2006; Angilletta, 2009). According to the 'thermodynamic-constraint' hypothesis, TPC may adapt to temperature by shifting towards T_{env} without changes in shape (Fig. 1a). According to the 'warmer is better' hypothesis, the shift of TPC may be accompanied by a decrease in maximum performance in cold-adapted populations (Fig. 1b). Some parameters of the TPC may vary independently from others, as adaptation may act only on some of them but not other. For example T_{opt} may change but not T_{min}, T_{max}, or maximum performance (Bennett & Lenski, 1993) (Fig. 1c).

Regarding competition we expect thermal adaptation to improve competitive ability at the temperature at which the species underwent long-term exposition. In this scenario, each species would reach higher densities if temperatures of origin and of destination matched than if they differed. Moreover, each species would reach higher densities when in presence of a competing species that is not pre-adapted to the destination temperature than when it is (Fig. 1d).

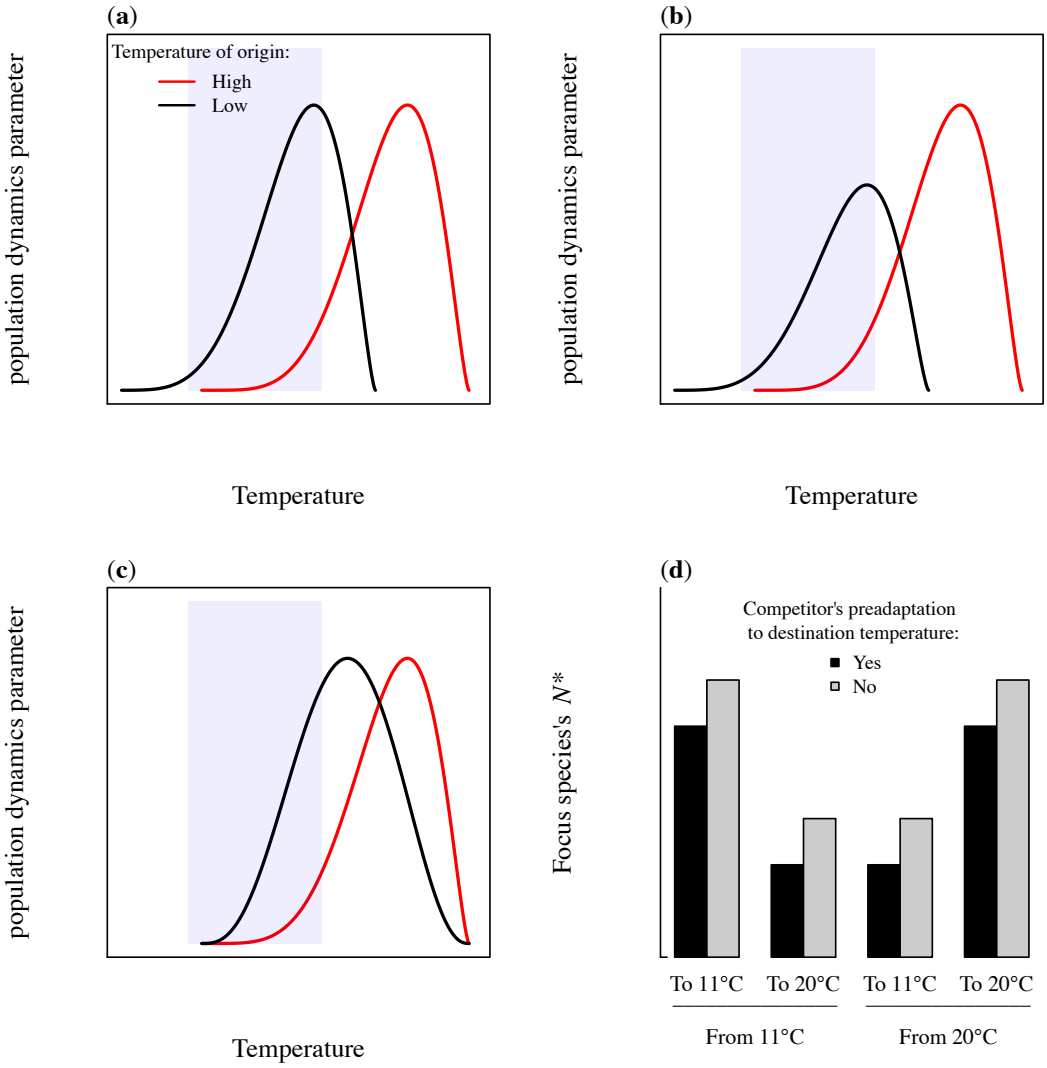

Figure 1. Possible outcomes for the single species dynamics experiment (a-c) and for the competition experiment (d). The shaded area in panels a-c represents the approximate temperature range under observation in the present study. Details are in the main text.

MATERIAL AND METHODS

The organisms used in this study are the free-swimming bacterivore ciliates *Paramecium caudatum* Ehrhard (hereafter *Paramecium*) and *Colpidium striatum* Stokes (hereafter *Colpidium*), obtained from the Carolina Biological Supply Company (CBS, Burlington, NC).

Long-term thermal adaptation

On October 3rd, 2011, monocultures of *Paramecium* were set up at 11°C, 20°C, and at 24.5°C, and monocultures of *Colpidium* were set up at 8°C, 11°C, and 20°C. We chose these temperatures because they are at the low and intermediate-high end of the temperature niche of the two species, at which both species can still maintain viable populations (Fig. S1 in the Supplementary information; *Chapter Two* of this Thesis). Both species were grown in monoculture in Protist Pellet medium 0.2g/L (Altermatt *et al*., 2015) inoculated with the bacteria *Serratia marcescens* and *Bacillus cereus* (also from CBS). Cultures were initially kept in 60×15 mm Petri dishes containing 14 mL of medium, established as part of a different experiment (*Chapter Two* of this Thesis). On December 15th, 2011, cultures were transferred to 100 mL jars. For the following ~22 months (≥ 130 generations depending on the temperature and species), stock cultures were maintained in the same temperature conditions, in jars of the same size. To ensure availability of nutrients and to avoid accumulation of catabolites, cultures were restarted every 3–5 weeks as follows. First, 1 L bottles of autoclaved Protist Pellet medium 0.2g/L were inoculated with *S. marcescens* and *B. cereus*. After inoculation, the medium was kept at 20°C for two days to allow bacterial growth, then, 95 mL of medium were poured in the culture jars, which were put in the incubators for one hour to allow acclimatization to the medium. Finally, 5 mL were transferred from the old stock cultures to the freshly prepared culture jars at the same temperature.

Experiment 1: effect of thermal adaptation on single-species dynamics

To assess whether long-term exposure to high or low temperatures affected how the single-species dynamics of the two species respond across a temperature gradient, we set up a translocation experiment. We set up monocultures of each species, from high and low temperatures, using the cultures from the long-term exposure experiment described above. We then transferred these to a gradient of five intermediate temperatures. From here onward,

we will refer to the temperatures at which the long-term stock cultures were kept as 'temperatures of origin', and the temperaures to which the cultures were transferred during the translocation experiment as 'destination temperatures' (Fig. 2).

On October 17th, 2013, the translocation experiment started. *Paramecium* cultures from each of the two temperatures of origin were set up at destination temperatures of 11°C, 15.5°C, 18.5°C, 20°C, and 24.5°C. *Colpidium* cultures from each of the two temperatures of origin were set up at destination temperatures of 8°C, 11°C, 15.5°C, 18.5°C, and 20°C. Temperature gradients were defined to be within a range at which both species can maintain viable populations. For all combinations of temperature of origin, destination temperature, and species, three experimental units were set up within each incubator. Incubators were dark during the whole experiment to minimize the chance of contamination by algae.

On day 0 of the translocation experiment, bacterized Protist Pellet medium 0.2g/L was poured into the experimental units, represented by 60×15 mm Petri dishes containing 10 mL of medium. Each experimental unit was inoculated with 40 individuals of the assigned species and placed in an incubator at its destination temperature.

Ciliate population densities were sampled every 24–48 hours until the end of the experiment, on day 21. Before each sampling, the volume of medium lost by evaporation was replaced with sterile, distilled water to maintain the concentration of dissolved salts constant. For sampling, each experimental unit was placed under a Hamamatsu C11440 camera, mounted on a Leica M205C microscope, and a 5 s digital film was recorded (25 frames per second). Depending on the cell density, the magnification used was 0.78x, 1.00x, 1.25x, or 1.60x, corresponding to surveying ~ 27.5, 16.3, 10.5, or 6.3% of the entire volume, respectively. The cell densities were estimated from the video files using the R package "bemovi" version 1.0 (Pennekamp *et al*., 2015).

To estimate the intrinsic growth rate, r and the total number of cells at approximate carrying capacity, $\hat{K}$ of *Paramecium* and *Colpidium* populations under each experimental condition, we fitted a logistic growth model to each abundance data series. We performed this with general maximum likelihood methods, using the function mle2 from the R package "bbmle" version 1.0.17 (Bolker & R Development Core Team, 2014). We use approximate carrying capacity, $\hat{K}$, instead of the actual carrying capacity, K, because during the experiment we did not maintain resources at a constant level. Although we did not replenish resources, most populations did not decline during the experiment; if they did, we estimated r and $\hat{K}$ using only the part of the time series preceding the decline. For each species we evaluated the

effects of temperature of origin, destination temperature, and their interaction on the observed values of r and $\hat{K}$.

To determine the correlation of r and $\hat{K}$ with temperature in the two species, the most parsimonious mixed effect model (with incubator identity as random effect to account for there being multiple experimental units in each incubator) was selected among the following models: one with interaction between origin and destination temperature, one without interaction (i.e. additive), and one with only destination temperature as fixed explanatory variable. When visual examination of plotted values suggested a non-linear dependence of the response variable on destination temperature, non-linearity was also assessed quantitatively by comparing the goodness-of-fit of models with polynomial and non-polynomial dependences. In case data resulted non-linearly correlated to temperature and visual examination suggested an exponential correlation, the exponent of the Arrhenius equation was estimated using a mixed-effect adaptation of the method used by Brown *et al*. (2004). The Arrhenius Equation is:

(Eqn. 1) $$I = i_0 \cdot e^{-E/kT}$$

Where:

- I is originally the rate of any chemical reaction, but in the context of the MTE it can also be any biological rate that depends on the metabolic rate, e.g. intrinsic growth rate;
- i_0 is the pre-exponential factor;
- E is an indirect estimate of the activation energy of the metabolic reactions which the biological rate in study depends on;
- k is the Boltzmann's constant;
- T is the environmental temperature in Kelvin.

Brown *et al*. (2004) estimated E by transforming Eqn. 1 as follows:

(Eqn. 2) $$log(I) = log(i_0) - E \cdot (1/kT)$$

We estimated the parameters of Eqn. 2 using a mixed effect model with incubator identity as random effect.

Mixed effect models were performed with function glmer of the R package "lmerTest" version 2.0-11 (Bates *et al.*, 2014; Kuznetsova *et al.*, 2015). In each model, a Gaussian or a Poisson distribution of residual errors was specified.

All statistical analyses were performed using R version 3.1.2 (R Core Team, 2015).

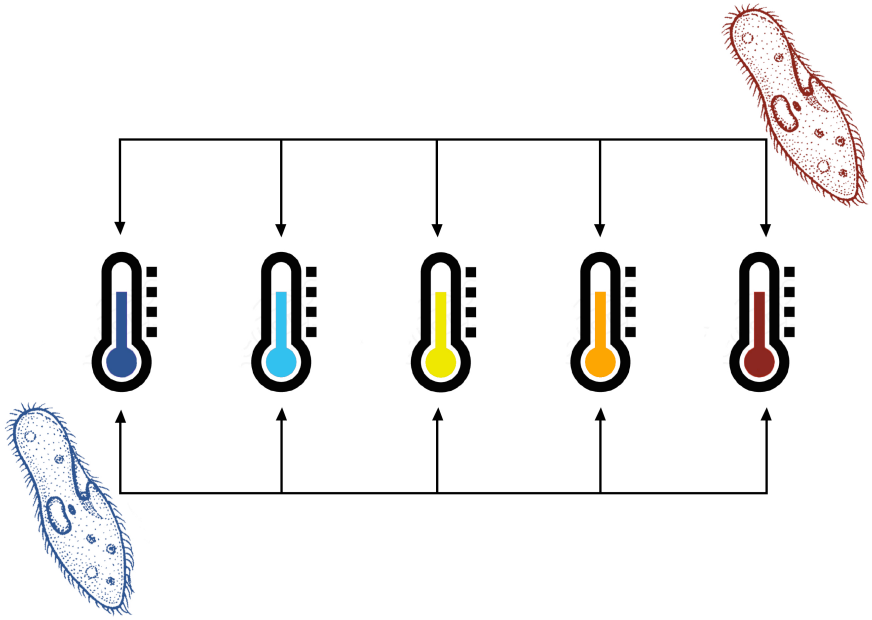

Figure 2. Design of Experiment 1, here shown for *Paramecium*. Blue and red *Paramecia* represent cold- and warm-adapted populations, respectively, and the thermometers represent the gradient of five temperatures, from low to high, at which population dynamics parameters were measured. The same design was adopted when using *Colpidium*, only the temperatures of origin and destination temperatures differed as specified in the main text. (*Paramecium* illustration by Ivy Livingstone, © BIODIDAC, http://biodidac.bio.uottawa.ca/).

Experiment 2: effect of thermal adaptation on interspecific competition

To assess whether long-term exposure to high or low temperatures affected the competitive ability of the two species at different temperatures, we performed a second translocation experiment, in which we chose 11°C and 20°C as both temperatures of origin and destination temperatures for both study species. Competition trials were set up for all the possible combinations of temperature of origin of each species and destination temperature (Fig. 3). For each combination, three microcosms were set up within each incubator. As before,

incubators were dark during the whole experiment to minimize the chance of contamination by algae.

Experimental cultures were set up following the same methodology as in Experiment 1, with the only difference that 40 individuals from each species were initially inoculated instead of 40 from only one species. Ciliate population densities were sampled every 24–48 hours until the end of the experiment, on day 21. Sampling consisted of withdrawing a small, known amount of medium from each microcosm and counting the number of individuals of each ciliate species in it under a dissecting microscope. Counting was not performed with the R package "bemovi" because the reliability of the cell counts in multispecies communities was not yet extensively tested at the time. After sampling, the withdrawn volume was returned to its experimental unit to minimize alterations of medium volume and species densities.

To estimate the total number of cells at equilibrium, N^* of *Paramecium* and *Colpidium* populations under each experimental condition, we fitted a logistic growth model to each abundance data series as described for Experiment 1. To estimate the competitive ability of each species in each experimental combination we compared its effect on the maximum density N^* of the other species. We used N^* instead of Lotka-Volterra competition coefficients (α_{ji}) because the latter require constant resources to be estimated reliably, while available resources were not kept constant during our experiment.

For each species we evaluated the effects of temperature of origin, destination temperature, pre-exposure of the competing species to the destination temperature (i.e. whether the temperature of origin of the competing species is the same as the destination temperature at which the competition trial was being carried out), and their interactions on N^*. We did so with a mixed-effect ANOVA with factor "microcosm identity" nested in the factor "destination temperature" as a random effect. Mixed effect models were performed with function glmer of the R package "lmerTest" version 2.0-11. In each model, a Poisson distribution of residual errors was specified.

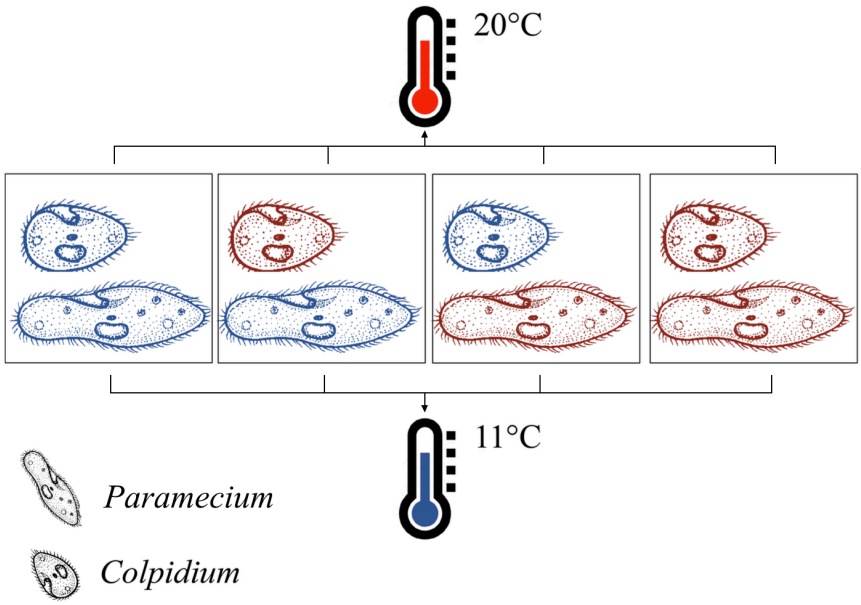

Figure 3. Design of Experiment 2. Blue and red *Paramecium* and *Colpidium* represent cold- and warm-adapted populations (11°C and 20°C), respectively, and the thermometers represent the temperatures at which competition trials were conducted. Each experimental combination was replicated three times (illustrations of *Paramecium* and *Colpidium* by Ivy Livingstone, © BIODIDAC, http://biodidac.bio.uottawa.ca/).

RESULTS

Experiment 1: effect of thermal adaptation on single-species dynamics

Temperature of origin affected the growth rate *r* of the two species in a species-specific manner: while the response of *Paramecium*'s *r* to temperature differed in populations from different temperatures of origin, *Colpidium*'s *r* responded to temperature in the same way regardless of the temperature from which the populations came from (Fig. 4a, b).

The growth rate of *Paramecium* increased non-linearly with temperature regardless of its temperature of origin, in a fashion described well by the Arrhenius equation. The growth rate of *Paramecium* coming from a long-term exposition at 11°C increased with temperature with an exponent of 1.00±0.07 (12 df, $t = 13.43$, $p < 0.0001$). Conversely, the exponent for *Paramecium* coming from a long-term exposition at 24.5°C was 0.65±0.10 (13 df, $t = 6.6$, $p < 0.0001$). This implies that *Paramecium* populations from 11°C reached a higher growth rate at 24.5°C (2.66±0.27 cells·cell^{-1}·day^{-1}) than populations of *Paramecium* from 24.5°C itself (1.34±0.12 cells·cell^{-1}·day^{-1}) (Fig. 4a). Conversely, at 11°C, *Paramecium* from 11°C and 24.5°C grew at similar rates, respectively 0.43±0.001 and 0.45±0.12 cells·cell^{-1}·day^{-1}. In the case of *Colpidium*, the temperature of origin did not affect the response of *r* to different temperatures. The growth rate of *Colpidium* populations from both 8°C and 20°C increased linearly by 0.05±0.001 cells·cell^{-1}·day^{-1}for each 1°C increase (27.97 df, $t = 11.92$, $p < 0.0001$) (Fig. 4b).

Thermal adaptation affected *Paramecium*'s $\hat{K}$, but not *Colpidium*'s. *Paramecium*'s $\hat{K}$ increased by 196±26 individuals per 1°C increase, regardless of the temperature which *Paramecium* came from (3.06 df, $t = 7.42$, $p = 0.0047$). Yet, *Paramecium*'s $\hat{K}$ was 316±129 cells higher in *Paramecium* from 24.5°C than in that from 11°C (22.97 df, $t = 2.4$, $p = 0.0227$), regardless of the destination temperature (Fig. 4c).

Colpidium's $\hat{K}$ displayed a hump-shaped temperature dependence with a maximum at ~15°C (Fig. 4d). The thermal response of $\hat{K}$ in cold-adapted and warm-adapted populations did not differ significantly (Table S1).

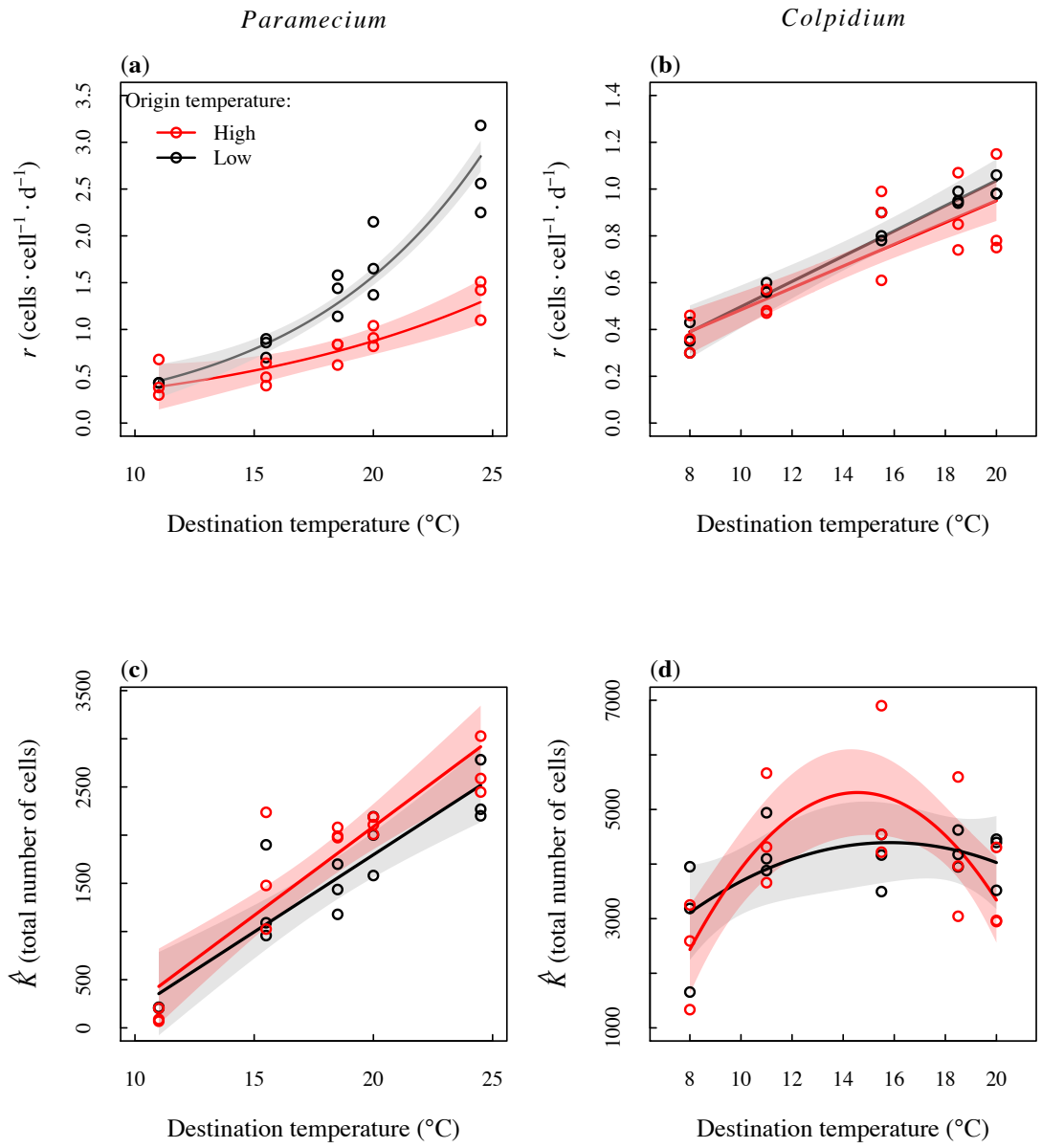

Figure 4. Effects of temperature of origin and of destination temperatures on *Paramecium*'s (a, c) and *Colpidium*'s (b, d) population dynamics. "High" and "low" temperatures of origin differ in the two species: they are 24.5°C and 11°C for *Paramecium* and 20°C and 8°C for *Colpidium*, respectively. Lines show predicted fits from the models. Shaded areas represent 95% confidence intervals. The thermal dependence of $\hat{K}$ does not differ significantly between cold- or warm-adapted *Colpidium* populations. Details in the main text and in Table S1.

Experiment 2: effect of thermal adaptation on interspecific competition

Paramecium and *Colpidium* differed in the way their competitive ability responded to the experimental treatments. While *Colpidium*'s pre-exposition to the competition trial temperatures did not affect *Paramecium*'s N^*, *Paramecium*'s pre-exposition to low or high temperature produced opposite effects on *Colpidium*'s N^* depending on the temperature at which the competition trial was carried out.

When competing with *Colpidium* pre-exposed to the temperature at which the trial was carried out, *Paramecium* did not reach lower N^* than when competing with non-pre-exposed *Colpidium*. On the contrary, *Paramecium*'s N^* showed a non-significant trend toward being higher in the presence of pre-exposed *Colpidium* (all p-values > 0.05, details in Table 1; Fig. 5a). This finding suggests that long-term pre-exposition to 11°C or 20°C did not give *Colpidium* competitive advantage at those temperatures.

The effect of *Paramecium* on *Colpidium*'s N^* depended on the temperature of origin and destination temperature of both species (Fig. 5b, Table 1). At 11°C, *Colpidium* from 11°C was positively affected by a competing species pre-exposed to the same temperature rather than a non pre-exposed competitor ($z = 2.09$, p-value = 0.0364). On the contrary, at 20°C, *Colpidium* from 11°C reached lower N^* when competing with *Paramecium* pre-exposed to 20°C rather than when the competing species came from 11°C ($z = -3.12$, p-value = 0.0018). *Paramecium*'s pre-exposition to the competition trial temperature did not change its competitive ability against *Colpidium* coming from 20°C, regardless of the temperature at which the competition trial was conducted (Table 1). These findings indicate that long-term pre-exposition of *Paramecium* to high temperature (20°C) increased its competitive ability against *Colpidium*, but only when the latter came from long-term pre-exposition to low temperature (11°C).

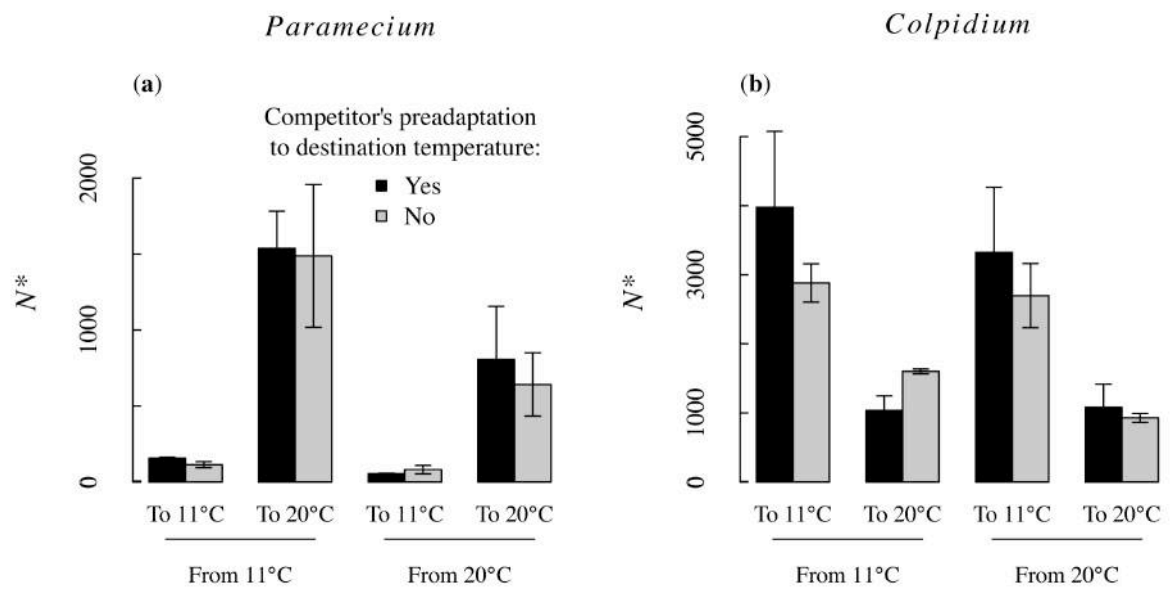

Figure 5. Outcome of the competition experiment for *Paramecium* (a) and *Colpidium* (b). On the x-axis, temperatures "from" and "to" refer to the temperatures of origin and destination of each species. The height of each column is proportional to the mean total number of cells (*N**) reached by the species in focus in each experimental treatment. Black columns refer to treatments in which the competing species was pre-exposed to the destination temperature, i.e. the competing species' temperature of origin was the same as the destination temperature at which the competition trial was being carried out. On the contrary, grey columns refer to treatments in which the competing species was not pre-exposed to the destination temperature.

Table 1. Pairwise contrasts between temperature treatments in which each species (*Paramecium* or *Colpidium*) was made to compete with a species (*Colpidium* or *Paramecium*) pre-exposed or not pre-exposed to the temperature at which the competition trial was carried out. "Temperature treatment" refers to the species the N^* of which is calculated. "Competitor's pre-exposure" tells whether the competing species' temperature of origin is the same as the competition trial destination temperature. The p-values indicates, for each temperature treatment, whether N^* is significantly different when the competitor was pre-exposed compared to when it was not.

Species	Temperature treatment	Competitor's pre-exposure	N^*	SE	z-value	p-value	
Paramecium	From 11°C to 11°C	No	113	1.15			
	From 11°C to 11°C	Yes	156	1.22	1.61	0.1077	
	From 20°C to 20°C	No	616	1.14			
	From 20°C to 20°C	Yes	755	1.21	1.06	0.2880	
	From 11°C to 20°C	No	1431	1.14			
	From 11°C to 20°C	Yes	1526	1.21	0.34	0.7341	
	From 20°C to 11°C	No	78	1.16			
	From 20°C to 11°C	Yes	53	1.24	-1.791	0.0734	
Colpidium	From 11°C to 11°C	No	2872	1.11			
	From 11°C to 11°C	Yes	3866	1.15	2.09	0.0364	*
	From 20°C to 20°C	No	925	1.11			
	From 20°C to 20°C	Yes	1050	1.15	0.88	0.3775	
	From 11°C to 20°C	No	1599	1.11			
	From 11°C to 20°C	Yes	1022	1.15	-3.12	0.0018	**
	From 20°C to 11°C	No	2669	1.11			
	From 20°C to 11°C	Yes	3222	1.15	1.32	0.1855	

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

DISCUSSION

Thermal adaptation differed between the two ciliate species, affecting intrinsic growth rates, approximate carrying capacity, and competitive ability in different ways.

Effect of thermal adaptation on single-species dynamics

For *Paramecium*, thermal adaptation affected how *r* responded to temperature changes. While the intrinsic growth rates of cold- and warm-adapted *Paramecium* did not differ at the low end of our experimental temperature gradient, the intrinsic growth rate of cold-adapted *Paramecium* was significantly stronger correlated with temperature than that of warm-adapted *Paramecium*. This result is compatible with a lowering of T_{opt} in cold-adapted *Paramecium*, possibly with other parameters of the TPC remaining unaffected. This finding suggests that TPC parameters may be subject to thermal adaptation to different degrees, as observed by both observational studies and laboratory-based experiments (Mongold *et al*., 1996; Frazier *et al*., 2006; Krenek *et al*., 2012). A study observing the thermal response of *Paramecium*'s *r* over the entire breadth of its TPC, allowing the quantification of all TPC parameters, is required to test this possibility.

Thermal adaptation also affected *Paramecium*'s $\hat{K}$. Warm-adapted *Paramecium* populations reached significantly higher $\hat{K}$-values than cold-adapted ones over the entire experimental gradient, while the slope of the correlation of $\hat{K}$ with temperature did not differ significantly between treatments. This may be explained by an adaptation to high population density mediated by temperature. Ancestral *Paramecium* populations reached higher maximum densities at higher temperatures (Fig. S1 in the Supplementary information). Warm-adapted *Paramecium* may have adapted to deal with more 'crowded' environments, possibly by reducing their cell size and/or by improving metabolic efficiency and thus extracting more energy from nutrients. This possibility could be tested by measuring changes in cell size and oxygen consumption, together with changes in $\hat{K}$ of cold- and warm-adapted *Paramecium*, along an experimental temperature gradient.

In contrast to *Paramecium*, neither *Colpidium*'s *r* or $\hat{K}$ were affected by long-term exposure to low or high temperatures. The striking contrast in the evolutionary adaptability of population dynamic parameters in *Paramecium* and *Colpidium* may be due to their genomes being subject to different mutation rates. These differences in the evolutionary potential of population parameters may reflect a different degree of adaptability of genetic sequences

underlying different life traits, such as rate of cellular division, cell mortality (both determining r), and metabolic efficiency (that affects $\hat{K}$). Examining this will require tracking changes in population dynamics together with the number of mutations accumulating in populations exposed to different temperatures. Holder & Bull (2001) provided an elegant example of this approach for bacteriophages, showing that changes in intrinsic growth rate were strongly correlated to the number of genetic mutations accumulated by the phage populations.

Effect of thermal adaptation on interspecific competition

The effects of thermal adaptation on competitive ability differed in *Paramecium* and *Colpidium*, and they contrasted with our expectation in both cases. Long-term pre-exposition of *Paramecium* to high temperature (20°C) increased its competitive ability at high temperature, as expected, but only against cold-adapted *Colpidium*. At low temperature, cold-adapted *Paramecium* facilitated *Colpidium*, in striking contrast with our expectations. We cannot provide an explanation for this. Beveridge *et al*. (2010a) observed that also predation increases the density of *C. striatum* at temperatures < 9°C. Although they could not explain this pattern either, it is possible that the positive effect of negative interspecific interactions (competition, predation) on the density of *Colpidium* at low temperature may have the same underlying mechanism. Contrary to the competitive ability of *Paramecium*, that of *Colpidium* was not affected by its pre-exposure to low or high temperatures.

The different degree of sensitivity of population and competition parameters to thermal adaptation suggests that species-specific trade-offs may exist, so that some parameters are subject to thermal adaptation more strongly than others. Further studies are needed to examine this possibility and to understand the underlying physiological and genetic mechanisms.

Conclusions

Our study shows that fast-growing ectotherms can adapt to environmental temperature over relatively short time scales (less than two years). Yet, even among species with comparable generation times, some can show greater adaptation than others. Moreover, thermal adaptation can affect population dynamics and interspecific interactions differently and in a species-specific fashion. This finding suggests that studies predicting climate-driven extinctions on

the basis of present-day responses to environmental temperature may picture a worse scenario than they would if adaptive ability to temperature was taken into account. A correlative study on the fly *Drosophila subobscura* supports this possibility. *Drosophila subobscura* is distributed in the Old and New World across a wide latitudinal range. With global warming, whithin less than three decades, genetic features that used to be typical of low, 'warm' latitudes have now spread into temperate latitudes (Balanya *et al*., 2006).Yet, our study shows that thermal adaptation can affect competition in counterintuitive and enigmatic ways, thus predicting the effect of climate change on interspecific interactions of adapting communities is not straightforward.

Our study confirms the potential of microcosm experiments for studying thermal adaptation not only at the level of genes, proteins, and individual life traits, but also at the ecological level of species both alone and interacting. Future studies should take advantage of microcosm experiments for expanding the study of adaptation of population and competition dynamics to temperature to include aspects of climate change other than mean temperature, such as temperature variability. Seminal studies in this direction have been undertaken by Lenski, Bennett, and colleagues (e.g. Bennett & Lenski, 1999). Moreover, future studies should address how pre-exposure to specific temperatures affects the temperature response in an integrated way, including population dynamics parameters together with individual phenotypic traits and genetic changes. This will clarify why and how some individual life traits and ecological parameters are subject to thermal adaptation more strongly than others and, in turn, will improve our ability to predict and manage the effect of climate change on population and community dynamics.

ACKNOWLEDGMENTS

Many thanks to Dr Frank Pennekamp for guiding us in the use of a pre-release version of package "bemovi".

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SUPPLEMENTARY INFORMATION

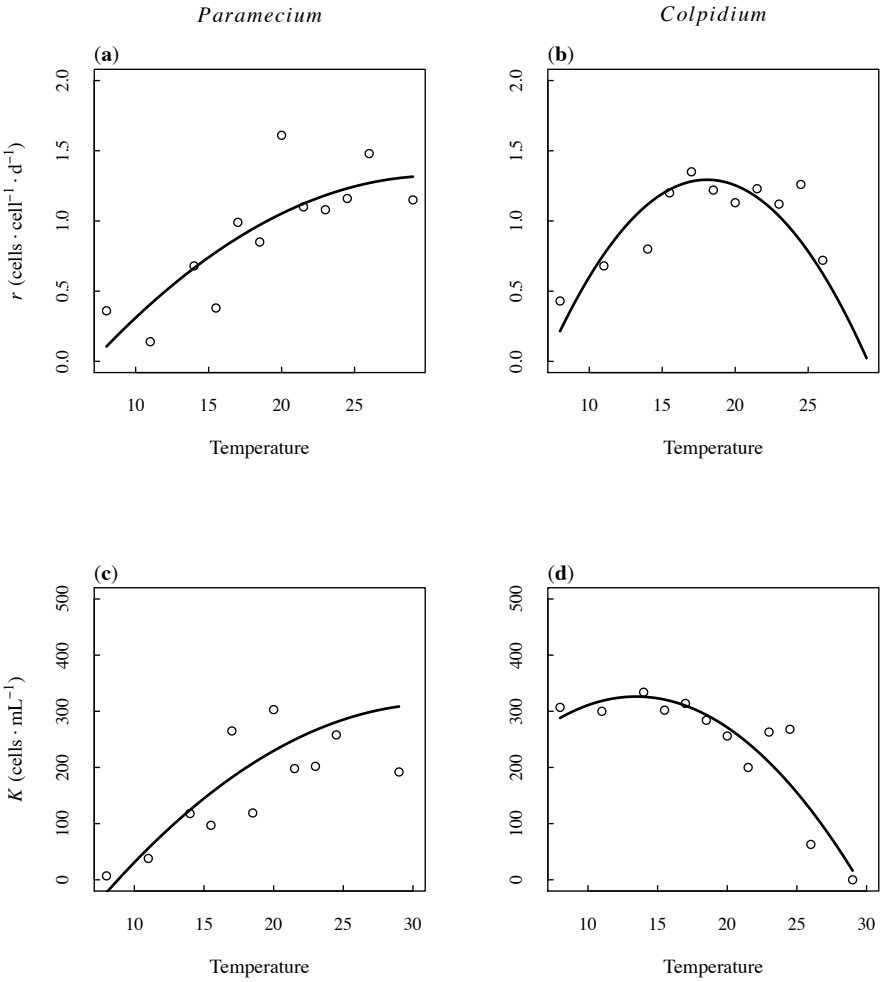

Figure S1. Estimates of growth rate, *r*, and density at carrying capacity, *K*, for *Paramecium* (a, c) and *Colpidium* (b, d) along a 12-step temperature gradient from 8°C to 29°C. These estimates were collected during a previous experiment (Plebani *et al*., 2015) set up in 60x15 mm Petri dishes containing 14 mL of medium analogous to that used in the present study.

Table S1. Temperature dependence of *Colpidium*'s $\hat{K}$ according to the model: $\hat{K} = \hat{K}_{15°C} + b \cdot T_{env} + c \cdot T_{env}^2$, where T_{env} is the destination temperature and is the estimated value of at 15°C.

Temperature of origin	Parameter	Estimate	SE	df	t value	p value
20°C	$\hat{K}_{15°C}$	3953	226.74	21.92	17.43	< 0.0001 ***
	a	1779.10	1241.92	21.92	1.43	0.1659
	b	-5024.16	1241.92	21.92	-4.04	0.0005 ***
8°C	$\hat{K}_{15°C}$	3936	320.66	21.92	-0.05	0.9567
	a	1700.94	1756.34	21.92	0.04	0.9649
	b	-1576.75	1756.34	21.92	1.96	0.0625

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

4

Substratum-dependent responses of ciliate assemblages to temperature: a natural experiment in Icelandic streams

(Manuscript published in Freshwater Biology, 60: 1561–1570*)*

Substratum-dependent responses of ciliate assemblages to temperature: a natural experiment in Icelandic streams

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Running headline: Temperature effects on ciliate assemblages

ABSTRACT

Ciliate assemblages play a significant role in the microbial food web. The effects of environmental temperature on assemblage composition may be influenced by abiotic factors such as seasonality and disturbance but the effects of temperature on ciliate assemblages found on different substrata have not been explored. Sandy bottoms and submerged rocks harbour dissimilar ciliate assemblages and it might be expected that their ciliate assemblages will respond differently to temperature. We studied how alpha diversity, beta diversity and total biomass of ciliate protist assemblages found on sandy bottoms and submerged rocks differed in 13 geothermally heated streams in Iceland whose mean temperatures range from 5–20 °C. We recorded number of operational taxonomic units (OTUs) and measured the size of cells in ciliate assemblages from both substrata. Effects of temperature on natural ciliate assemblages were substratum-dependent. On rock surfaces both total ciliate biomass and alpha diversity declined with increasing temperature, and beta diversity increased with increasing temperature difference due to OTU nestedness (assemblages from warm streams being composed chiefly of subsets of the OTUs found in colder streams). In sandy substrata, however, ciliate assemblage composition was independent of temperature. Substratum-specific responses may be due to differences in mechanical disturbance, nutrient availability or exposure to invertebrate grazers. Rock-surface assemblages may be more exposed to the flow and retain less nutrient than those of sandy substratum; thus they may be more strongly resource-limited and more responsive to direct effects of temperature on metabolism. Alternatively, rock surface assemblages may be more exposed to grazing by invertebrates, which intensifies with temperature. Our study highlights the need to account for environmental context such as substratum type to fully understand the effect of temperature on microbial assemblages in streams. Future increases in global temperatures may affect freshwaters differently depending on their prevalent substratum. Those dominated by hard substrata may have their ciliate assemblages, and thus food-web structures and ecosystem functioning, more strongly affected by warming relative to systems dominated by soft substrata.

Keywords: biomass; ciliates; diversity; natural experiment; temperature gradient.

INTRODUCTION

Temperature plays a fundamental role in driving ecological processes (Kordas, Harley & O'Connor 2011; Sibly, Brown & Kodric-Brown 2012) and its effects on one or a few species have received considerable attention in recent years (Montagnes, Kimmance & Atkinson 2003; Vasseur & McCann 2005; Vucic-Pestic *et al*. 2011; Lang, Rall & Brose 2012). Community-level effects such as changes in alpha and beta diversity, however, are still largely unknown (Woodward, Perkins & Brown 2010b), and most studies have focused on large-scale observations over latitudinal or altitudinal gradients, or smaller-scale warming experiments (Stewart *et al*. 2013). Latitudinal gradients of alpha and beta diversity have been documented for many taxa, and temperature is often invoked as the major explanatory variable (Buckley & Jetz 2007). But in contrast to the observational studies, experiments have revealed conflicting and contingent community-level effects for grasses (Yang *et al*. 2011; Lamb *et al*. 2011; Sherry *et al*. 2012; Wahren *et al*. 2013; Post 2013), fungi and bacteria (Lamb *et al*. 2011) and larger eukaryotes (Villalpando et al., 2009; Bakonyi et al., 2007; Strecker et al., 2004; Meerhoff et al., 2012). Given the criticisms that can be levelled at either approach (confounding variables in surveys, and a lack of realism in experiments) research that incorporates both the realism of observational studies and the lack of confounding variables in experimental studies is needed to reconcile these apparently contrasting findings at the community level (O'Gorman *et al*. 2014).

Here, we focus on the effect of temperature on freshwater benthic assemblages of ciliate protists (hereafter 'ciliates'). Ciliates respond promptly to environmental change (Jiang & Morin 2004) and can be extremely diverse within small areas (Finlay & Esteban 1998; Esteban & Finlay 2007), which makes them an ideal model system for studying community-level relationships between environmental temperature and biodiversity. Moreover, ciliates play significant roles in the microbial food web and the cycling of organic material, for example, by feeding on bacteria and increasing decomposition rate (Ribblett, Palmer & Coats 2005; Kathol *et al*. 2009), contributing to the import of organic material from the water column into the benthos (Eisenmann *et al*. 2001; Weitere, Schmidt-Denter & Arndt 2003; Kathol *et al*. 2009) and being consumed by invertebrates (Norf, Arndt & Weitere 2007).

Previous studies have shown that temperature can increase the total biomass and species richness of ciliate assemblages and induce changes in their beta diversity (Norf & Weitere 2010; Vidussi *et al*. 2011; Domaizon *et al*. 2012). Furthermore, the direction and magnitude of temperature effects can change depending on environmental factors such as

seasonality (Norf *et al*. 2007), nutrient availability (Norf & Weitere 2010) and mechanical disturbance (Marcus *et al*. 2014). The nature of the bottom substratum is also known to affect the abundance and species composition of ciliate assemblages (Gucker & Fischer 2003). Yet, to our knowledge, no-one has assessed whether ciliate assemblages found on dissimilar substrata respond differently to a temperature gradient. Given the significant ecological role played by ciliates, understanding the potentially interacting effects of temperature and substratum type on their biomass and diversity is important to predict the effects of climate change on a local scale. In our study, we assessed the effect of temperature on the total biomass, alpha diversity and beta diversity of freshwater ciliate assemblages found on two contrasting substrata, namely submerged rock surfaces and sandy sediment.

The study was carried out in a network of small streams characterised by a natural and stable geothermal gradient spanning about 15 °C (Friberg *et al*. 2009). This system allows us to evaluate long-term effects of temperature on community composition and organism abundance in a natural experiment, without the confounding effects of biogeography associated with altitudinal or latitudinal gradients (O'Gorman *et al*. 2014). Previous studies carried out in the same system have found some clear effects of temperature: macrofaunal community similarity decreases with increasing pairwise temperature difference between streams, and primary productivity and decomposition rates increase with temperature, whereas effects on the non-algal microbial community are still largely unknown (Friberg *et al*. 2009; Woodward *et al*. 2010a).

We specifically tested the hypotheses that biomass and alpha diversity of ciliate assemblages are related to environmental temperature as well as substratum. We expect both biomass and alpha diversity to decrease with increasing temperature, because the high metabolic costs linked to high temperature would lead to stronger competition (Brown *et al*. 2004; Savage *et al*. 2004; Binzer *et al*. 2012). We also tested the hypothesis that beta diversity among assemblages increases with increasing temperature difference, as previously observed in mesocosm experiments (Norf & Weitere 2010; Domaizon *et al*. 2012). Furthermore, we assessed the relative contributions of taxa turnover and nestedness to variation in composition. Finally, we tested the hypothesis that substratum type affects the responses of ciliate assemblages to environmental temperature. We expected different substratum types to have different levels of resource availability and to be exposed to different levels of mechanical disturbance. These factors affect the structure of the ciliate assemblages (e.g. Foissner & Berger 1996), and could influence how ciliate assemblages respond to temperature differences (Norf & Weitere 2010; Marcus *et al*. 2014). We expected ciliate assemblages on

submerged rock surfaces to be more resource-limited than on sandy substratum and thus predict a stronger reduction in biomass and alpha diversity with increasing temperature on rock compared to sandy substratum.

METHODS

Study site

Our study was conducted in 13 streams in a single geothermal catchment in Hengill, about 30 km east of Reykjavík, Iceland (64°.03'N: 021°.18'W; 350-420 m a.s.l.) (Fig. S1), displaying a gradient of mean water temperatures from 5.4 to 20.2°C. Although there are some minor chemical differences among the streams, these are independent of temperature, which is the major environmental gradient in the system (Table S1) (Woodward *et al*. 2010a; Demars *et al*. 2011; O'Gorman *et al*. 2012; Adams *et al*. 2013).

Ciliate sampling

During August 2012 we surveyed ciliate assemblages in 13 streams (Fig. S1), from two different substrata in each stream: submerged rock surfaces and coarse sandy sediment (hereafter 'sandy substratum'). Three to four samples from each substratum were taken from each stream. All samples in a stream were collected from locations at known coordinates, and analyzed within 24 hours of collection. Sandy substratum samples were collected by scooping about 15 ml of sediment into a 50-ml sterile plastic tube and topping up the rest of the tube volume with natal stream water. Samples from submerged rock surfaces were collected by haphazardly selecting a rock and removing it from the stream; the size of all rocks was approximately 350 cm^3 (the size of a fist). The upper surface of each rock was brushed with a clean toothbrush, photographed and measured to allow standardization of cell abundances per unit area. Brushed material was washed off the toothbrush into a 50-ml sterile plastic tube using a squirt bottle filled with natal stream water, until the tube contained 50 ml.

In the laboratory, the contents of each vial were homogenised by gently shaking the tubes and 1 ml of water was withdrawn (following O'Gorman *et al*. 2012). This procedure was chosen because it homogenises the samples without damaging the cells of free-living ciliates. The withdrawn volume was poured into a Sedgewick-Rafter cell counter and

searched using a Nikon Eclipse E200 compound microscope at 100–400× magnification. Ciliates were identified *in vivo* to the lowest possible taxonomic level, termed operational taxonomic units (hereafter OTUs) using identification keys (Foissner *et al*. 1991; Foissner & Berger 1996; Patterson & Hedley 2009); in most cases, an OTU corresponds to a genus. The number of individuals of each OTU per millilitre was recorded, and the length and width of each cell were measured *in vivo* to the nearest ten micrometres using ocular micrometres.

Length and width measurements of each individual were used to estimate the corresponding volume. This was performed by approximating the cell shape of each OTU to standard geometric forms (O'Gorman *et al*. 2012, after Hillebrand *et al*. 1999) and estimating the corresponding volume. Individual cell masses were obtained by multiplying their estimated volumes by their physical density. The density of each cell was taken to be 1 g cm^{-3}, the same as distilled water, as water is the major component of eukaryotic cells (Omori & Ikeda 1984, Kageyama *et al*. 1989). Total ciliate biomass per millilitre was calculated as the sum of the masses of all ciliate cells found per millilitre surveyed.

Temperature data

Stream temperature was recorded between 7 and 21 August 2012. Streams 2–4, 10 and 12 were surveyed using 'iButton' data loggers (Maxim Integrated, San Jose, California, USA), which recorded temperature once every 20 minutes (precision ±0.1°C). Streams 5–9, 11, 13 and 14 were surveyed using 'HOBO Pro v2' and 'HOBO Pendant' temperature loggers (Onset Computer Corporation, Cape Cod, Massachusetts, USA), which recorded temperature at 15-minute intervals (precision ±0.2°C). All statistical analyses were performed using the mean of the temperatures recorded over the sampling period as the explanatory variable.

Statistical analyses

Statistical analyses were performed in R version 3.0.3 (2014). Multivariate analyses were performed with the R packages vegan version 2.0-10 (Oknasen et al. 2013) and betapart version 1.3 (Baselga et al. 2013). Rock- and sand-communities required different sampling techniques and methods of standardizing ciliate densities (individuals per unit area versus individuals per unit volume). Thus they were analyzed separately, as differences in sampling would have confounded the results of joint analyses (e.g. ANCOVA).

Alpha diversity. Alpha diversity was measured as OTU richness (i.e. the number of OTUs found per sample). The relationship between temperature and ciliate OTU richness was assessed separately for each substratum type, using generalised linear mixed effects models (hereafter GLMMs) with stream temperature as a fixed effect and stream identity as a random-intercept effect to account for variability among streams due to factors other than temperature. We used a Poisson error distribution to account for the nature of the data (integer count data). The models were implemented with the 'glmer' function from the package lme4 version 1.1-7 (Bates *et al*. 2014), and the relative contributions of stream temperature and stream identity to R^2 were estimated with the 'r.squaredGLMM' function from the package MuMIn version 1.10.0 (Barton 2014).

Beta diversity. Total pairwise beta diversity was measured using the *Sørensen* index, an effective tool to highlight broad compositional patterns (Gotelli & Ellison 2004). The *Sørensen* index is based on OTU presence/absence, and represents a more robust approach than abundance-based dissimilarity indices when the assumption of species abundances being measured without bias may not be met (Baselga *et al*. 2013), as is the case in our study. It measures beta diversity due to both OTU nestedness and OTU turnover. We followed the approach proposed by Baselga *et al*. (2013) to disentangle these two components. For each pair of ciliate assemblages, the beta diversity due to OTU turnover was measured with the Simpson index, while the beta diversity due to OTU nestedness was computed as the difference between the *Sørensen* index and the Simpson index (Baselga 2010). This was performed using function 'beta.pair' of the R package betapart version 1.3 (Baselga *et al*. 2013).

Mantel tests were used to assess the correlation between beta diversity components (total, due to OTU nestedness and due to OTU turnover) and temperature differences among streams. Although effective and widely used, the Mantel test has been suggested to be at high risk of incurring type I errors (incorrect rejection of a true null hypothesis) (Guillot & Rousset

2013); moreover, Mantel tests cannot detect non-linear correlations. To account for these limitations, we also assessed the correlation between each beta diversity component and temperature by means of non-metric multidimensional scaling ordinations (nMDS), arguably the most robust unconstrained ordination method in community ecology (Oknasen *et al*. 2013), combined with two-dimensional generalised additive models (GAM) (Simpson 2011; Battarbee *et al*. 2012). This approach consisted of two steps. First, the relative beta diversity distances among assemblages were displayed in two dimensions using ordinations. The outcome of the ordination was then used as a response variable in a GAM, with mean stream temperatures as the explanatory variable. The analysis was performed with the function 'ordisurf' in R package vegan version 2.0-10, which computes the significance of the GAM and allows the model fit to be visualised as an overlay on the nMDS plot (as smoothing isoclines).

Total biomass. The relationship between temperature and ciliate biomass was assessed separately for each substratum type using linear mixed effects models (hereafter LMMs), with stream temperature as a fixed effect and stream identity as a random-intercept effect. We used natural-log-transformed biomass as the response variable to account for the positive-only nature of biomass values. The models were implemented with the 'lmer' function from the package lmerTest 2.0-6 (Kuznetsova, Brockhoff & Bojesen-Christensen 2014), and the contributions of stream temperature and stream identity to R^2 were estimated with the 'r.squaredGLMM' function from the package MuMIn 1.10.0 (Barton 2014).

RESULTS

In total we identified 6,018 live individuals from 83 OTUs. Of these, 2,705 individuals of 48 OTUs were found in the samples from rock surfaces and 3,313 individuals from 72 OTUs in the samples from sandy substrata; 38 OTUs were common to both substrata (Fig. S2–S3).

Effects of temperature on alpha diversity

The number of OTUs declined significantly with increasing temperature on the rock surfaces (exponent: -0.068 per degree C°, z-test = -4.808, $p < 0.0001$) and temperature explained 41.6% of this variability (Fig. 1a). The number of OTUs found in assemblages from sandy substratum was not correlated with temperature (exponent: 0.011 per degree C°, z-test =

0.627, p = 0.53, $R^2_{temperature}$ = 0.008) (Fig. 1b). For both substrata, the amount of variability among streams explained by temperature was larger than that explained by stream identity (i.e. reasons other than temperature *per se*), but smaller than the residual variability due to either natural heterogeneity or the observation process (Table S2).

Effects of temperature on beta diversity

Total beta diversity in ciliate assemblages from neither substratum was correlated with changes in environmental temperature (Fig. S4a,b; Fig. S5a,b; Table 1). Similarly, the component of beta diversity due to OTU turnover was unrelated to temperature in either substratum (Fig. S4e,f; Fig. S5e,f; Table 1). The Mantel test (Fig. 2a) and the GAM model run on an nMDS ordination (Fig. S5c) showed that nestedness-related beta diversity of rock-surface assemblages increased linearly with increasing pairwise temperature difference (Table 1). Conversely, ciliate assemblages from sandy substratum did not differ due to taxonomic nestedness across the temperature gradient (Fig. 2b; Fig. S5d; Table 1). In light of the observed negative relationship between OTU richness and temperature (see previous section), this result suggests that, on rock surfaces, the OTU composition of ciliate assemblages from warm streams is nested within the OTU composition at lower temperatures (i.e. ciliate assemblages from warm streams are subsets of those from the colder streams).

Total ciliate biomass

The total ciliate biomass on rock surfaces significantly declined with increasing temperature (slope = -0.177, t-test = -2.891, p = 0.0209) (Fig. 3a). Temperature explained 22.7% of the observed variability among streams. Total ciliate biomass found in sandy substratum did not show a significant correlation with temperature (slope = -0.0150, t-test = -0.265, p = 0.793) (Fig. 3b). Most of the variability not explained by temperature was due to within-stream variability (Table S3) (i.e., either to natural heterogeneity or the observation process).

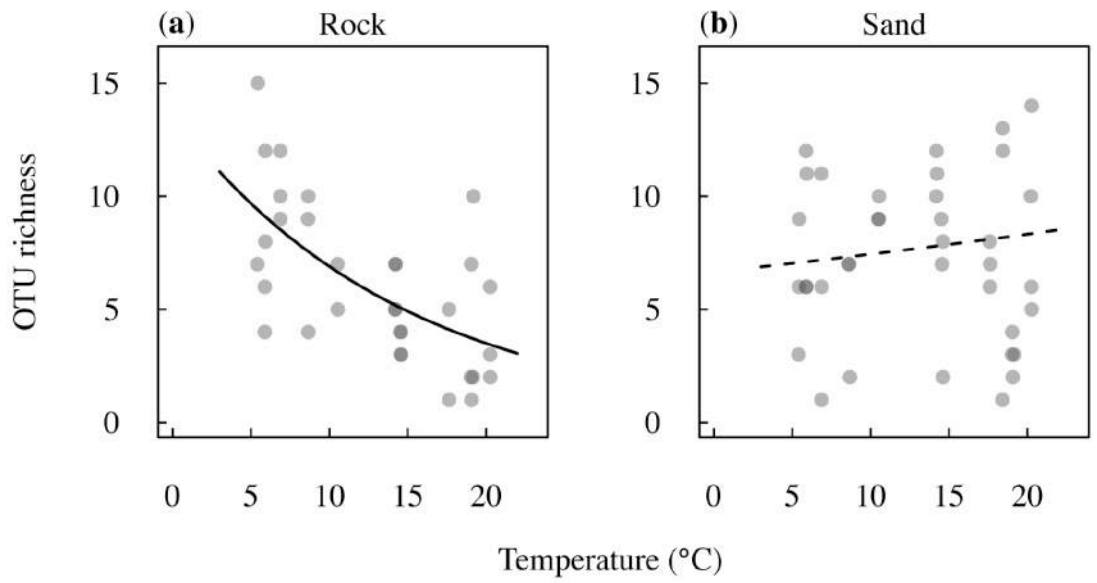

Figure 1. Relationship between temperature and alpha diversity (taxon richness) in ciliate assemblages found on (a) submerged rock surfaces and (b) sandy substrata. Solid and dashed lines represent statistically significant relationships and non-significant trends, respectively.

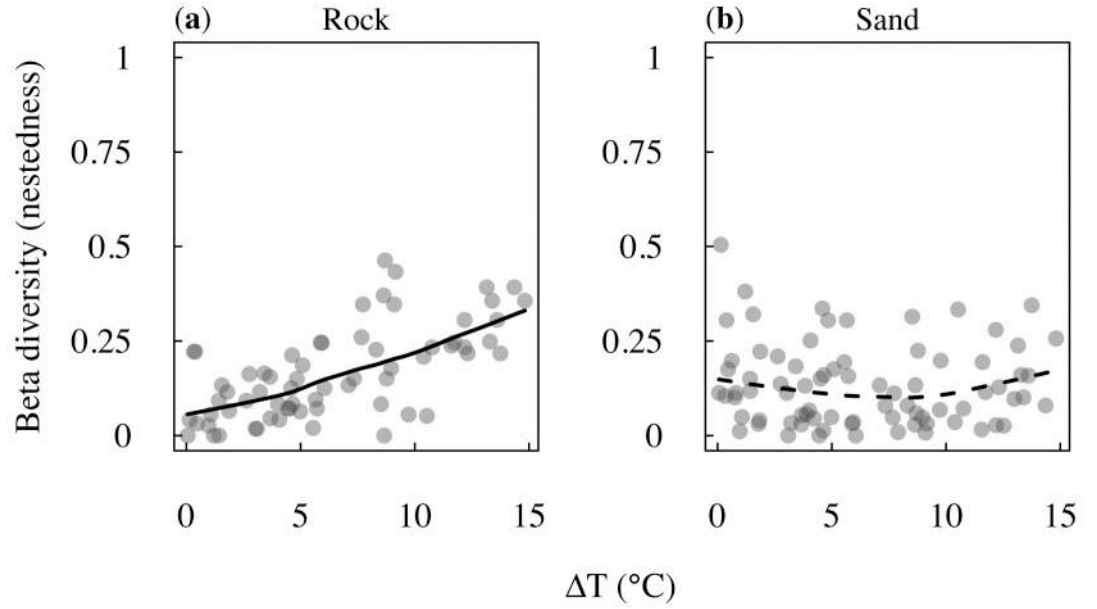

Figure 2. Correlations between the pairwise temperature difference between streams (ΔT) and the corresponding pairwise differences in beta diversity due to nestedness, for ciliate assemblages found on (a) submerged rock surfaces and (b) sandy substrata.

Table 1. Results of Mantel tests and GAM models, aimed at detecting linear and non-linear relationships between beta diversity and temperature, respectively. Mantel's r is based on Pearson's product-moment correlation, and p is the corresponding p-value computed over 999 permutations. GAM's R^2 is the adjusted r-squared value; p is the approximate significance of smooth terms based on an F-test.

Substratum	Beta diversity component	Mantel test		GAM		
		r	p	R^2	F	p
Rock	Total (Sørensen)	-0.03	0.591	0.287	0.492	0.225
	Nestedness	0.66	0.001 **	0.768	4.042	0.0003 ***
	Turnover	-0.44	0.998	0	0	0.793
Sand	Total (Sørensen)	-0.1	0.804	0.165	0.263	0.291
	Nestedness	-0.06	0.652	0.371	0.786	0.0871
	Turnover	-0.04	0.632	0	0	0.491

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

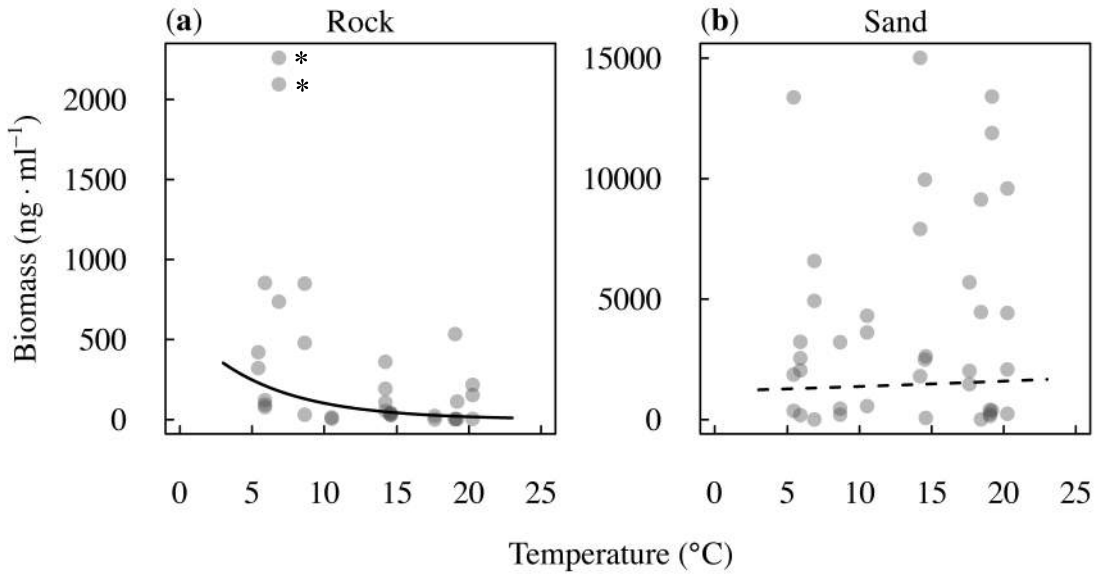

Figure 3. Correlation between temperature and total ciliate biomass (ng ml^{-1}) found on (a) submerged rock surfaces and (b) sandy substrata. Solid and dashed lines represent statistically significant relationships and non-significant trends, respectively. Data outliers in the figure are represented by asterisks (*).

DISCUSSION

Our goal was to assess the relationship of temperature with the alpha diversity, beta diversity and total biomass of natural ciliate assemblages on different substrata. To the best of our knowledge, ours is the first study to do so. The role of temperature depended on the nature of the substratum. In assemblages from submerged rock surfaces, alpha diversity, beta diversity due to nestedness and total biomass were all significantly affected by temperature, while none of these relationships were observed in sandy substratum.

The negative correlation of ciliate alpha diversity and total biomass with temperature on submerged rock surfaces was in line with our expectation that high temperatures increase metabolic demands (Brown *et al*. 2004; Savage *et al*. 2004; Binzer *et al*. 2012), thus increasing competition and potentially reducing carrying capacity. However, on sandy substratum these measures were not correlated with temperature. This could have several explanations. Sandy substratum may retain more nutrients than rock surfaces because of more interstitial space (Hossain *et al*. 2014), and the two substratum types may be exposed to different regimes of mechanical disturbance due to water flow. Both resource availability and disturbance can change the direction and magnitude of temperature effects on assemblages (Norf & Weitere 2010; Marcus *et al*. 2014). Moreover, sandy substrata may differ depending on grain size or composition and differential retention of fine organic material. Thus, the diversity and abundance of ciliate communities from sandy substrata may be more strongly correlated with sand grain size or composition than to temperature. Sandy substratum in the Hengill system, mostly coarsely-grained lava sediment, shows some differences in grain size among streams, but we lack quantitative measurements to assess the relationship between sediment granulometry and the diversity and abundance of ciliate communities.

Temperature may also influence protist assemblages indirectly, affecting the strength of interactions among species. Temperature increases the metabolic requirements of individuals (Brown et al. 2004) and, consequently, it can increase their feeding rates (e.g. Sanford 1999, Yee & Murray 2004). Changes in the intensity of trophic interactions can, in turn, alter the dynamics and composition of whole communities (Paine 1974). Biofilm-grazing gastropods represent common consumers or competitors of ciliates, and temperature is known to increase both the feeding rate and the activity of gastropods (Yee & Murray 2004). In our study system, *Radix balthica* snails play a key role in the food web as the dominant grazer in the warmer streams (O'Gorman *et al*. 2012). *Radix balthica* consumes the same resources (bacteria and diatoms) as many protists, and it is more abundant and exerts

higher grazing pressure on the biofilm at high temperatures (O'Gorman *et al*. 2012). The increased abundance and grazing pressure of *R. balthica* at high temperatures may reduce species richness and total biomass in ciliate assemblages, either by direct competition for resources, by physically displacing ciliates while grazing the biofilm or by consuming them directly. Moreover, an effect of grazing might be more substantial on a firm substratum such as a rock surface than on shifting sandy substratum. Unfortunately, there is no experimental evidence in the literature either supporting or rejecting these possibilities. We could not assess or distinguish between these possible explanations within the scope of this study, and further work is needed to detect the ecological processes underlying the patterns we observed. For example, an exclusion experiment would make it possible to determine the relative importance of temperature and biofilm grazers such as *R. balthica* on ciliate assemblages on different substrata (Trochine *et al*. 2011).

On submerged rock surfaces, temperature differences caused an increase in ciliate beta diversity due to taxonomic nestedness, while ciliate beta diversity due to taxonomic turnover was not affected. This suggests for submerged rock surfaces that changes in the beta diversity of ciliate communities are due to environmental filtering exerted by temperature, more than to niche partitioning of ciliate taxa along the temperature gradient (Baselga 2010). As temperature increases, it may act as an 'environmental filter', progressively excluding more and more ciliate taxa from submerged rock surfaces. This role may be a direct reflection of ciliate niche breadths, or it may be mediated by trophic interactions. In the former case, the taxonomic nestedness of ciliate assemblages at different temperatures suggests that ciliates found on rock surfaces are similarly tolerant to low but not to high temperatures. Conversely, sandy-substratum assemblages may include more 'temperature-generalist' ciliates, explaining why temperature did not drive changes in their beta diversity. In the latter case, resource competition with invertebrate grazers such as *R. balthica* may also explain the progressive exclusion of species from low to high temperatures in rock-surface assemblages, but not in the sandy-substratum. None of the taxa we identified are classified as psychrophilic (cold-specific) or thermophilic (Foissner *et al*. 1991). Further studies are required to disentangle whether the environmental filtering role of temperature is directly related to the niche breadth of the taxa or trophically mediated, and whether ciliates from sandy substrata share any traits, such as large cell size or phenotypic plasticity, that would enable them to cope with such broad temperature gradients.

The different effect of temperature on the components of beta diversity is a novel and important finding, because previous studies have only tested its influence on total beta

diversity, without evaluating whether the observed patterns were due to changes in taxonomic turnover or taxonomic nestedness.

We acknowledge some limitations and possible sources of bias in our study. Since samples need to be identified live, such studies are forced to employ a relatively low sampling intensity (three to four samples for each combination of stream and substratum type), which may not have been enough to reach taxonomic saturation (i.e., rare species may be under-represented in our dataset). A higher sampling effort in future studies would show whether our results also apply when all rare species are accounted for. A second possible source of bias is represented by the procedure for sampling sandy substrata. While the gentle stirring of the samples allowed homogenization while minimizing cell damage, it may have led to underestimation of the abundance of some ciliate groups, such as those living attached to the substratum (e.g. stalked perithrichs). Future studies should use the same technique we used for sampling delicate, free-swimming ciliates, paired with a more vigorous shaking of the samples to ensure the detachment of ciliates attached to the substratum.

Our study highlights that differences in environmental context, such as substratum type, must be taken into account if we are to understand and predict the effect of temperature on microbial diversity and biomass. In the context of ongoing climate change, predicted increases in global temperatures may produce locally different effects on ciliate assemblages depending on their substrata. Specifically, ciliate assemblages found on submerged rock surfaces may be more prone to undergo changes in diversity and total biomass than ciliate assemblages from sandy substrata. We might expect to see larger changes in systems dominated by hard substrata (e.g., upland ecosystems), than in those dominated by soft or sandy substrata (e.g., lowland ecosystems). In turn, this may produce substratum-dependent changes in microbial food-web structure and ultimately in ecosystem functioning. Thus, substratum-dependent changes in the response to temperature at a microscopic level may ultimately be manifested at far larger spatial scales.

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SUPPORTING INFORMATION

Table S1: Table showing the values for abiotic variables for each stream, ordered by mean stream temperature. Temperature data were collected during 7th–21st August 2012. Abiotic variables other than temperature were collected in August 2008 for all streams but Stream 2, for which the data refer to Summer 2004 (data from Adams et al. 2013 and Woodward et al. 2009, respectively). Temp = mean temperature in °C; pH is unitless; Cond = conductivity in μS cm^{-1}; all other environmental parameters are given in mg l^{-1}. DOC = dissolved organic carbon; DO = dissolved oxygen; TN = total nitrogen; TP = total phosphorous.

Stream	Temp	pH	Cond	DOC	DO	NH_4	NO_3	TN	PO_4	TP	Ca^{2+}	K^+	Mg^{2+}	$Na2^+$	Si	Cl^-	SO_2^{4-}
10	5.43	7.7	129	0.314	11.7	0.006	0.002	0.054	0.010	0.018	20.1	0.3	4.7	11.0	6.0	6.6	3.8
7	5.89	7.6	110	0.208	11.4	0.010	0.001	0.012	0.012	0.025	14.1	0.5	4.1	10.6	7.0	7.7	1.5
13	6.86	7.6	201	0.294	11.1	0.018	0.001	0.114	0.001	0.006	28.4	0.5	8.9	15.1	9.9	7.2	5.9
11	8.65	8.0	624	0.581	9.9	0.009	0.001	0.085	0.001	0.015	32.4	2.5	29.5	123.8	25.4	5.7	0.2
14	10.52	8.1	254	0.403	10.7	0.010	0.003	0.123	0.001	0.010	35.9	0.5	8.5	17.5	11.4	6.4	5.8
9	14.20	8.1	262	0.263	8.5	0.008	0.004	0.036	0.018	0.036	26.9	1.5	6.6	34.6	17.9	6.6	2.7
12	14.53	7.9	223	0.618	8.1	0.012	0.005	0.098	0.001	0.011	25.1	1.4	7.7	19.6	17.6	7.0	2.6
4	14.59	7.7	153	0.465	10.7	0.008	0.006	0.041	0.001	0.008	20.3	0.3	5.1	7.4	9.8	7.6	2.9
5	17.62	8.0	282	0.427	8.0	0.006	0.006	0.032	0.002	0.019	31.1	2.2	6.7	36.8	19.8	6.3	4.8
2	18.42	7.9	236	NA	10.6	NA	NA	NA	0.013	0.019	NA	NA	NA	NA	NA	NA	14.4
3	19.04	7.9	275	0.226	7.1	0.013	0.004	0.019	0.009	0.028	29.5	1.8	6.5	29.9	19.6	6.4	6.4
6	19.18	8.1	283	0.317	8.6	0.011	0.002	0.016	0.007	0.028	29.8	2.2	6.4	37.0	19.7	6.5	4.6
8	20.25	8.1	300	0.330	6.8	0.009	0.006	0.014	0.012	0.031	28.2	2.6	5.5	37.5	20.2	6.3	3.5

Table S2. Results of the GLMMs assessing the correlation between temperature and alpha diversity (OTU richness) in ciliate assemblages found on rock surfaces and sandy substrates. $R^2_{(temperature)}$ and $R^2_{(Stream)}$ represent the proportion of total variation explained by temperature and stream identity, respectively.

Substrate	Temperature effect	SE	Test	p	$R^2_{(temperature)}$	$R^2_{(Stream)}$
Rock	-0.06776	0.01409	z = -4.808	<0.0001***	0.416	0
Sand	0.01061	0.01691	z = 0.627	0.53	0.008	0.004

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Table S3. Results of the LMMs assessing the correlation between temperature and the natural log of total ciliate biomass (originally in ng/ml) found on rock surfaces and sandy substrates. $R^2_{(temperature)}$ and $R^2_{(Stream)}$ represent the proportion of total variation explained by temperature and stream identity, respectively. ([1]): outliers included.

Substrate	Temperature effect	SE	Test	p	$R^2_{(temperature)}$	$R^2_{(Stream)}$
Rock	-0.2133	0.0685	t = -3.113	0.0128 *	0.2886	0.1131
Rock[1]	-0.1770	0.0612	t = -2.891	0.0209 *	0.2273	0.0086
Sand	0.0150	0.0566	t = 0.265	0.793	0.0018	0

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

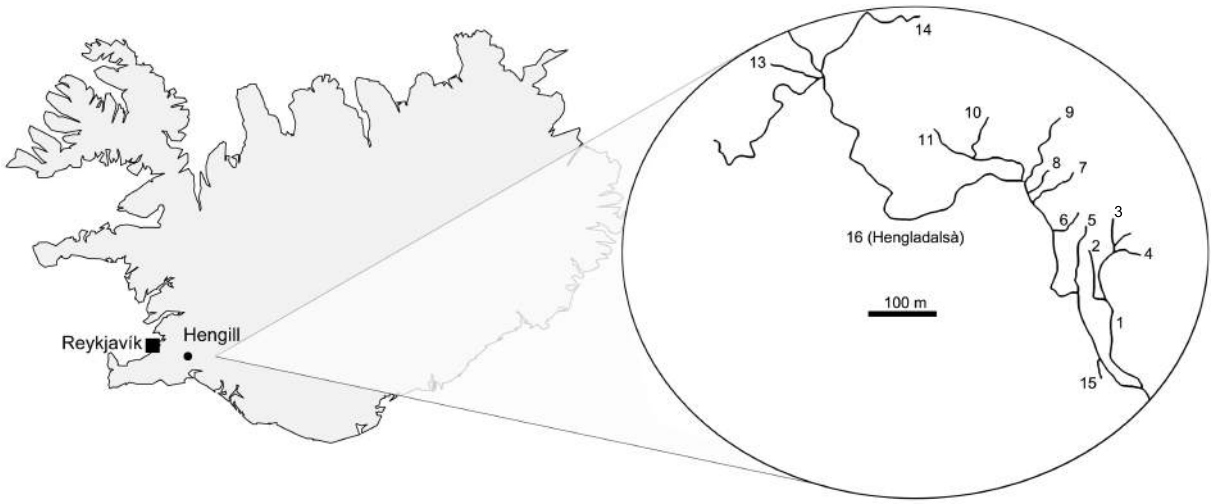
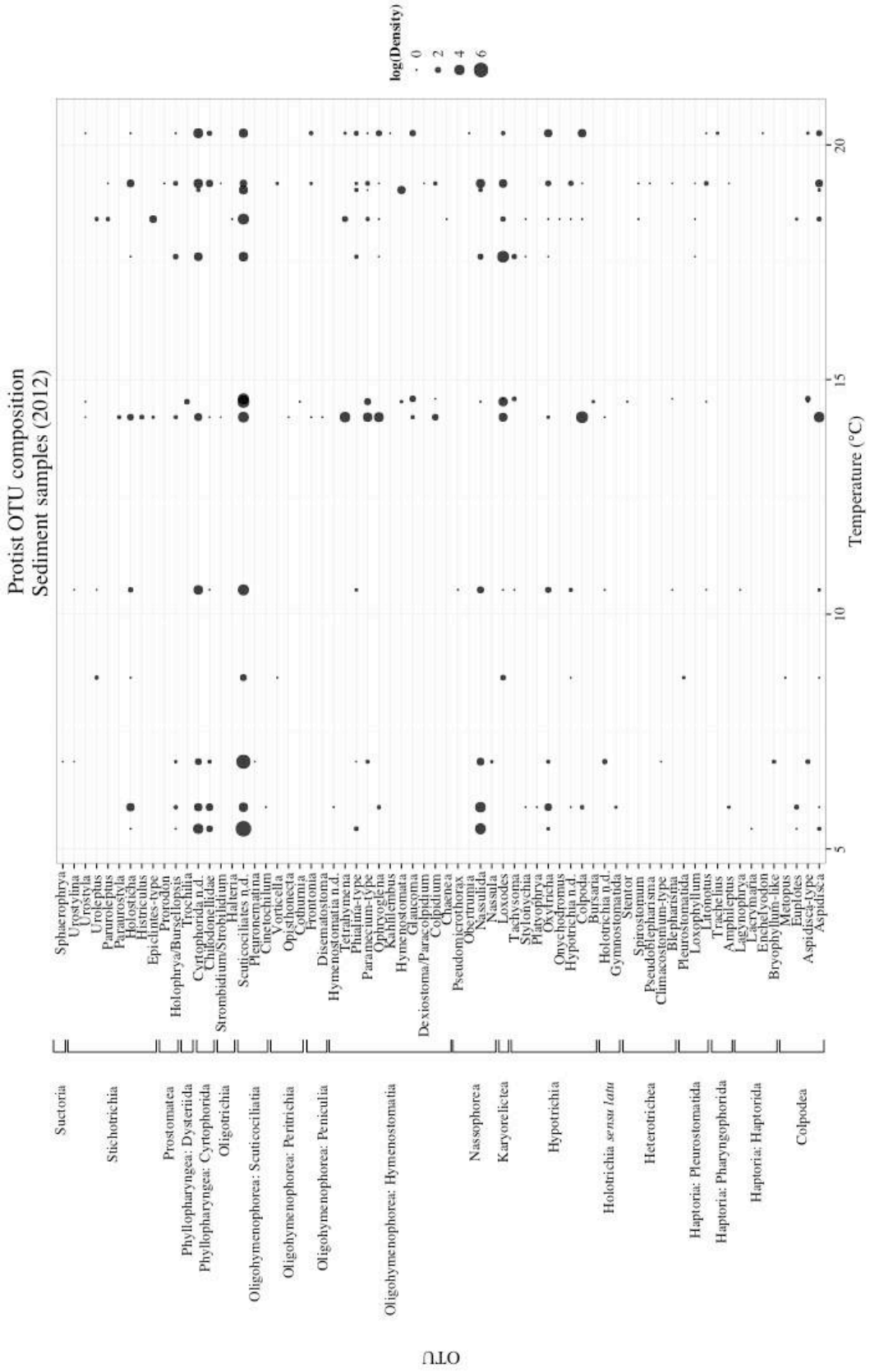


Figure S1. Location of Hengill in Iceland and map of the stream network. Streams 1, 15, and 16 were not included in the study. Stream 12, not shown, is located about 1.5 km south-east of Stream 15.

Figure S2 (page 101). Protist OTU present in the samples from soft sediment collected at each temperature.

Figure S3 (page 102). Protist OTU present in the samples from rock surfaces collected at each temperature.


Protist OTU composition
Sediment samples (2012)
log(Density)
0
2
4
6
Temperature (°C)
5
10
15
20
OTU
Sphaerophrya
Urostylina
Urostyla
Uroleptus
Paruroleptus
Paraurostyla
Holosticha
Histriculus
Epiclintes-type
Prorodon
Holophrya/Bursellopsis
Trochilia
Cyrtophorida n.d.
Chilodonellidae
Strombidium/Strobilidium
Halteria
Scuticociliates n.d.
Pleuronematina
Cinetochilum
Vorticella
Opisthonecta
Cothurnia
Frontonia
Disematostoma
Hymenostomatia n.d.
Tetrahymena
Philaina-type
Paramecium-type
Ophryoglena
Kahliellinus
Hymenostomata
Glaucoma
Dexiostoma/Paracolpidium
Colpidium
Chaenea
Pseudomicrothorax
Obertrumia
Nassulida
Nassula
Loxodes
Tachyssoma
Stylonychia
Platyophrya
Oxytricha
Onychodromus
Hypotrichia n.d.
Colpoda
Bursaria
Holotrichia n.d.
Gymnostomatida
Stentor
Spirostomum
Pseudoblepharisma
Climacostomum-type
Blepharisma
Pleurostomatida
Loxophyllum
Litonotus
Trachelius
Amphileptus
Lagynophrya
Lacrymaria
Enchelyodon
Bryophyllum-like
Metopus
Euplotes
Aspidisca-type
Aspidisca
Suctoria
Stichotrichia
Prostomatea
Phyllopharyngea: Dysteriida
Phyllopharyngea: Cyrtophorida
Oligotrichia
Oligohymenophorea: Scuticociliatia
Oligohymenophorea: Peritrichia
Oligohymenophorea: Peniculia
Oligohymenophorea: Hymenostomatia
Nassophorea
Karyorelictea
Hypotrichia
Holotrichia sensu lato
Heterotrichea
Haptoria: Pleurostomatida
Haptoria: Pharyngophorida
Haptoria: Haptorida
Colpodea

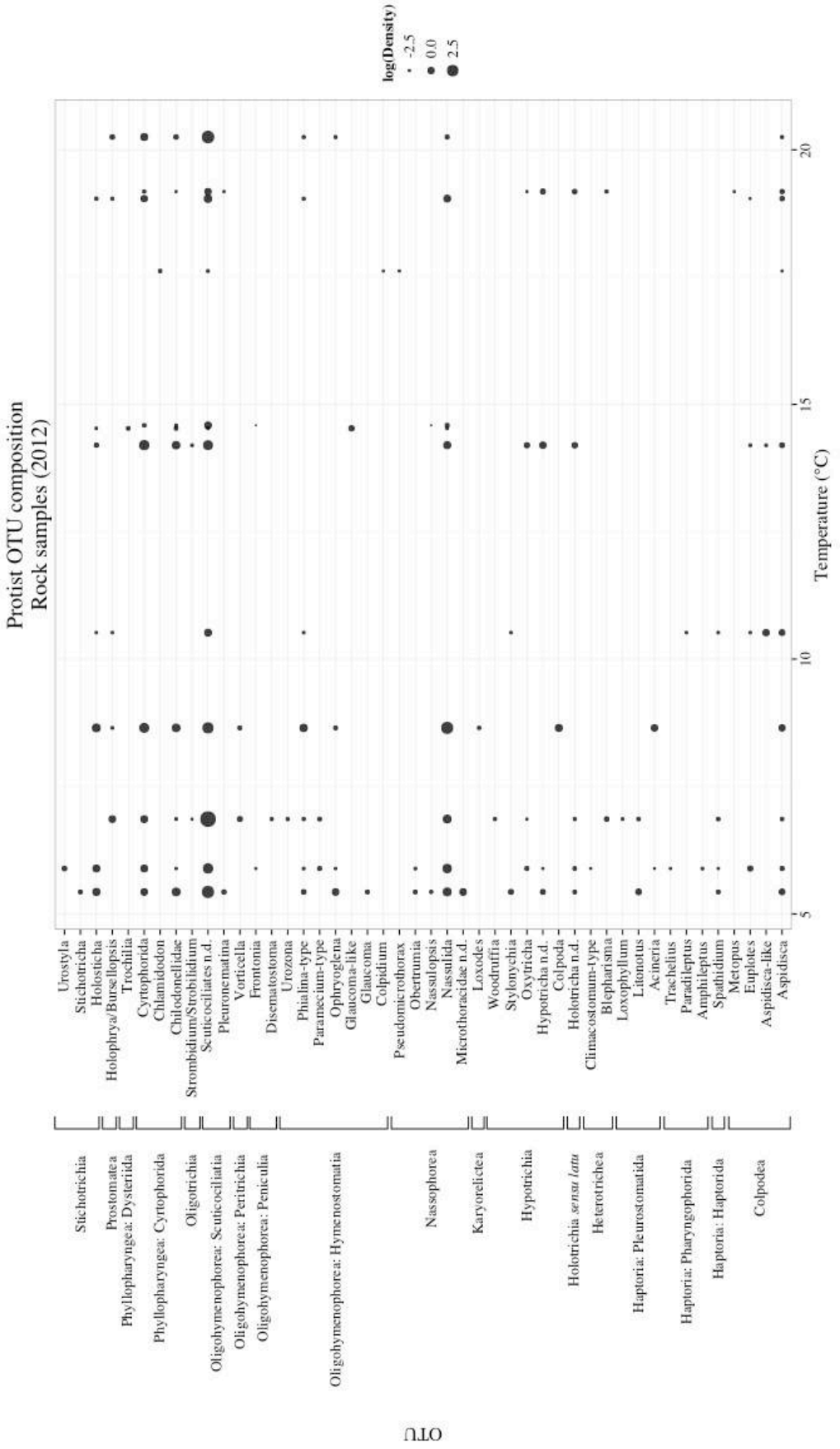
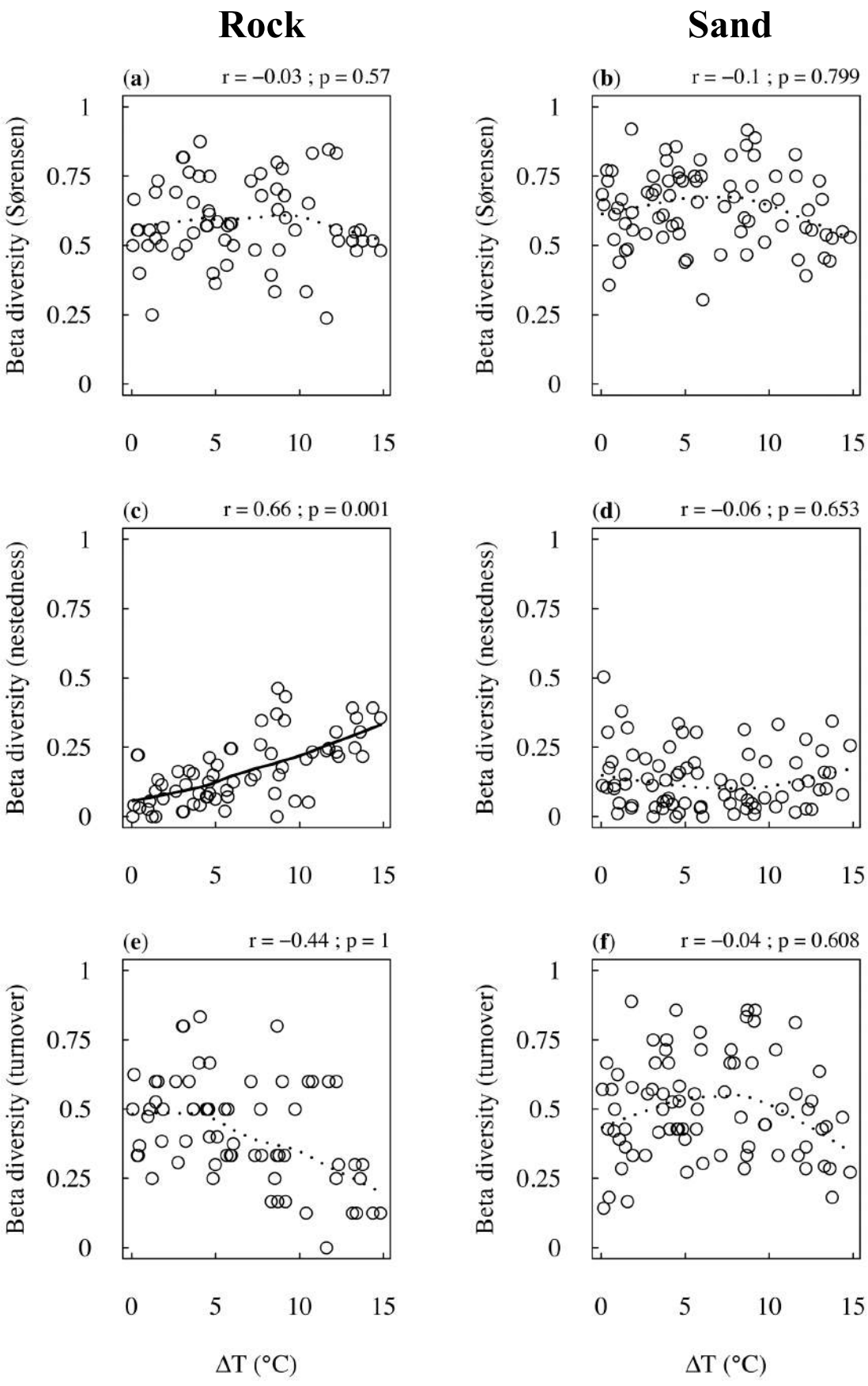
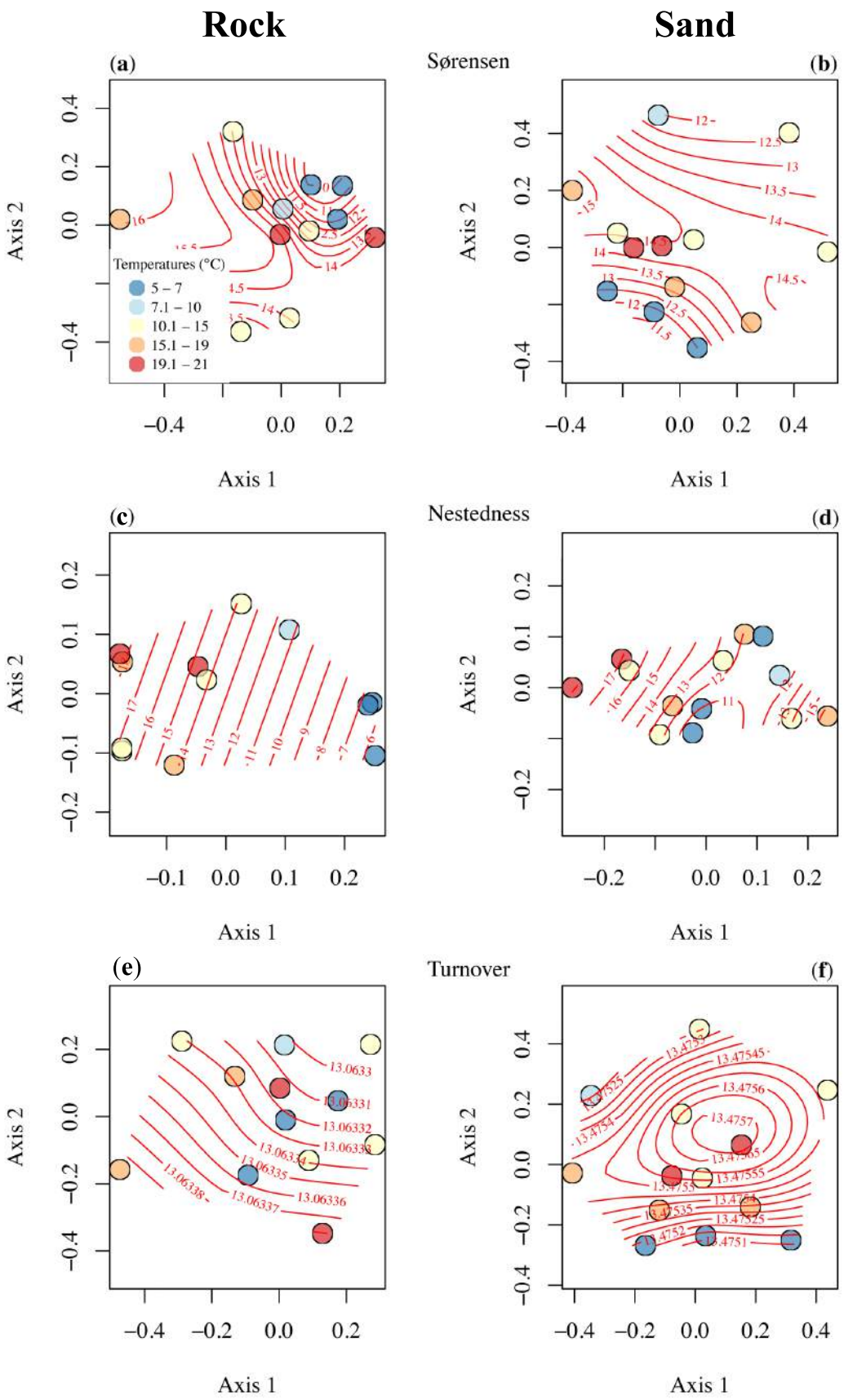

Protist OTU composition
Rock samples (2012)
log(Density)
-2.5
0.0
2.5
Temperature (°C)
5
10
15
20
OTU
Urostyla
Stichotricha
Holosticha
Holophrya/Burselloposis
Trochilia
Cyrtophorida
Chlamidodon
Chilodonellidae
Strombidium/Strobilidium
Scuticociliates n.d.
Pleuronematina
Vorticella
Frontonia
Disematostoma
Urozona
Phialina-type
Paramecium-type
Ophryoglena
Glaucoma-like
Glaucoma
Colpidium
Pseudomicrothorax
Obertrumia
Nassulopsis
Nassulida
Microthoracidae n.d.
Loxodes
Woodruffia
Stylonychia
Oxytricha
Hypotricha n.d.
Colpoda
Holotricha n.d.
Climacostomum-type
Blepharisma
Loxophyllum
Litonotus
Acineria
Trachelius
Paradileptus
Amphileptus
Spathidium
Metopus
Euplotes
Aspidisca-like
Aspidisca
Stichotrichia
Prostomatea
Phyllopharyngea: Dysteriida
Phyllopharyngea: Cyrtophorida
Oligotrichia
Oligohymenophorea: Scuticociliatia
Oligohymenophorea: Peritrichia
Oligohymenophorea: Peniculia
Oligohymenophorea: Hymenostomatia
Nassophorea
Karyorelictea
Hypotrichia
Holotrichia sensu latu
Heterotrichea
Haptoria: Pleurostomatida
Haptoria: Pharyngophorida
Haptoria: Haptorida
Colpodea

Figure S4 (page 104). Correlations between the pairwise temperature difference between streams (ΔT, in °C) and the corresponding pairwise differences in beta diversity for ciliate assemblages found on rocky (panels a, c, e) and sandy substrates (panels b, d, f). On top of each panel, the corresponding outcome of a Mantel test is reported; r is based on Pearson's product-moment correlation and p is the corresponding p-value computed by bootstrapping over 999 permutations.

Figure S5 (page 105). Non-linear Multi Dimensional Scaling (nMDS) ordinations based on the differences in beta diversity for ciliate assemblages found on rocky (panels a, c, e) and sandy substrates (panels b, d, f). The isoclines represent a smooth surface obtained by fitting a two-dimensional GAM model having the nMDS ordinations as a response variable and the mean stream temperatures as explanatory variable. The only significant correlation is that between temperature and the nestedness component of beta diversity in rock assemblages (F test, $p = 0.0003$; $r^2 = 0.768$) (c).


Rock
Sand
(a)
r = −0.03 ; p = 0.57
(b)
r = −0.1 ; p = 0.799
(c)
r = 0.66 ; p = 0.001
(d)
r = −0.06 ; p = 0.653
(e)
r = −0.44 ; p = 1
(f)
r = −0.04 ; p = 0.608
Beta diversity (Sørensen)
Beta diversity (nestedness)
Beta diversity (turnover)
0
0.25
0.5
0.75
1
0
5
10
15
ΔT (°C)


Rock
Sand
(a)
Sørensen
(b)
Temperatures (°C)
5 – 7
7.1 – 10
10.1 – 15
15.1 – 19
19.1 – 21
(c)
Nestedness
(d)
(e)
Turnover
(f)
Axis 1
Axis 2

5

Do ectotherms get smaller as temperature increases? A test on the micro-invertebrates of a geothermal stream network

(Manuscript in preparation)

Do ectotherms get smaller as temperature increases? A test on the micro-invertebrates of a geothermal stream network

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Running headline: Temperature effects on ectotherm size

ABSTRACT

Ectotherm body size exhibits a negative correlation with temperature in the majority of laboratory-based studies, but the studies assessing the generality of this correlation in nature are few, hindered by confounding factors, and give contrasting results. Almost all living species are ectotherms, and body size and temperature play a key role in affecting their ecology. Thus, assessing the generality of the negative temperature–size correlation among ectotherms in nature is crucial for the understanding of ecological dynamics. We examined the temperature–body size relationships of benthic, heterotrophic ciliate protists, shelled amoebae, nematodes, and rotifers in a network of geothermally heated streams in Iceland. We first compared the mean size of the different organism groups sampled from submerged rocks from five streams whose natural mean temperatures range from 5 to 20 °C. Furthermore, we performed a warming experiment to compare the mean body size of organisms from the warmed section of a stream (9-10 °C) to that of organisms from the unwarmed section of the same stream (6 °C). In this experiment we also assessed whether the effect of temperature on body size is influenced by the substrate where the organisms are found (submerged rock surfaces or sandy substrate). In the naturally heated streams, only the size of Pseudomicrothoracida ciliate protists displayed a significant, negative correlation with temperature. In the warming experiment, only shelled amoebae from sandy substrates were significantly smaller in the warmed section of the stream compared to the unwarmed section. Our results suggest that the negative temperature–body size relationship among ectotherms may be less common in nature than expected based on laboratory-based studies. We propose that temperature variability in nature may be sufficient to prevent general effects of mean temperature on body size. Alternatively, species body size may respond differently to temperature depending on their temperature niche optimum. These possibilities require further assessment to test whether declining body size is a universal response to warming, and to understand how to account for it in models predicting the ecological effects of climate change.

Keywords: freshwater, Iceland, Temperature–Size Rule, protists, rotifers, nematodes.

INTRODUCTION

Body size has a major effect on the physiology, performance, and fitness of organisms. Similarly, environmental temperature affects almost all biological rates, with consequences for body size itself (Kingsolver & Huey, 2008). Together, body size and body temperature play a key role in influencing the ecology of organisms, primarily by affecting their energetic requirements (Brown *et al*., 2004). Understanding the relationship between environmental temperature, body temperature, and body size is essential for correctly predicting the ecological effects of current global climate change.

Geographical gradients in body size have long been known for endotherms, which tend to be larger at higher, 'cold' latitudes (Bergmann, 1847; Meiri & Dayan, 2003). This pattern, known as Bergmann's rule, is explained by the selective advantages that different body sizes confer in different climates. Large bodies have a low surface-to-volume ratio that helps to preserve body heat, an advantage in cold climates. By contrast, the high surface-to-volume ratio of small bodies facilitates heat loss, a favorable trait in warm climates (Edeline *et al*., 2013).

Ectotherms also tend to display a negative correlation between environmental temperature and body size (Ray, 1960; Atkinson, 1994). Although it is usually described at the level of species (Atkinson's 'Temperature–Size Rule'), this negative temperature–body size relationship can be expected to manifest itself at several, non-mutually exclusive levels of biological organisation (Daufresne *et al*., 2009). If the relationship has general validity across species, it should lead to a decrease in mean body size with increasing temperature at the community scale. This overall pattern may be due to an increase in the proportion of small species in the community, and/or to a reduction in mean body size at the population (intra-specific) level. The latter may be due to a general decrease in individual body size, or to an increase in the proportion of juveniles in the population.

There is no consensus on which mechanism causes the inverse temperature–body size correlation in ectotherms, or even on whether a single mechanism can explain this pattern for all ectotherms regardless of taxon and habitat (Atkinson & Sibly, 1997). The heat preservation hypothesis proposed for endotherms does not generally apply to ectotherms, which are mostly thermoconformers, thus various alternative explanations have been suggested. The negative relationship between

temperature and ectotherm body size may be mediated by trophic interactions. For example, the strength of competition and predation generally increases with temperature (Brown *et al*., 2004), and this may lead to an increase in the selective advantage of small-bodied competitors and prey (Zaret, 1980; Edeline *et al*., 2013). The negative temperature–body size correlation in ectotherms may also arise from physiological effects. One hypothesis suggests that cell division rate increases with temperature faster than cell growth rate does. The mismatch between cell division and cell growth rates would increase with temperature, thus resulting in smaller, faster-growing organisms in warmer climates (van der Have & de Jong, 1996). An alternative, physiology-related hypothesis that has recently gained attention suggests that the negative temperature–body size correlation in ectotherms results from differences in oxygen availability at different temperatures (Forster *et al*., 2012; Horne *et al*., 2015). Aerobic metabolism has a stronger positive relationship with temperature compared to the diffusional uptake of oxygen, hence oxygen availability becomes more limiting as temperature increases. A large ratio between respiratory surfaces and body volume facilitates oxygen uptake, favoring small-bodied organisms at higher temperatures. According to this hypothesis, differences in the strength of the relationship in different ectotherm groups may be due to their different oxygen requirements. In particular, negative temperature–size correlation should be steeper in aquatic environments, where oxygen content is much lower than in air, and where the high viscosity and high density of water makes oxygen uptake more energy-demanding. Moreover, large-bodied organisms should decrease in size with increasing temperature at a faster pace than small-bodied organisms, because of their high surface-to-volume ratio (Forster *et al*., 2012).

Although ectotherms represent > 99% of living species, relatively few studies have investigated ectotherm temperature–size correlations and their underlying mechanisms. Most studies supporting the negative temperature–size correlation in ectotherms focus on species reared individually in laboratory conditions (Ray, 1960; Atkinson, 1994). Out of 109 studies on ectotherm animals, plants, protists, and a bacterium, 91 (83.5%) showed negative temperature–size correlations (Atkinson, 1994). Field studies assessing whether this general pattern also holds in natural conditions have produced contrasting evidence. Fossil data suggest that soil-burrowing fauna (insects, oligochaeta, molluscs, crayfish) became smaller during the Paleocene-Eocene Thermal Maximum, supporting the general role of temperature as a

driver of ectotherm body size (Smith *et al*., 2009). Similarly, both freshwater and sea fish decreased in body size over the last three decades, a trend which may be due to the increase in mean temperatures (Daufresne *et al*., 2009). On the other hand, according to a study of North American freshwater fish, only warm-water species tend to decrease in size with decreasing temperature, while cold-water species display the opposite trend (Rypel, 2014). Studies based on latitudinal/altitudinal gradients gave heterogeneous, taxon-specific results, with anuran amphibians, turtles, a sea snail, and diatoms becoming smaller with increasing latitude/altitude, lizards, snakes, and urodele amphibians presenting the opposite pattern, and land snails and most insects showing no clear pattern (Trussell, 2000; Ashton & Feldman, 2003; Hausdorf, 2003; Finkel *et al*., 2005; Shelomi, 2012).

Field studies have the merit of assessing the validity of the temperature–size relationship for ectotherms in nature, on larger spatio-temporal scales than laboratory experiments. However, they are hindered by confounding factors that are difficult to control, and which have been suggested to produce effects on body size. For example, the dwarfism of soil-burrowing fauna during the Paleocene-Eocene Thermal Maximum could have been driven by dry conditions or low quality vegetal food as well as by temperature (Smith *et al*., 2009), the decrease in fish body size over the last three decades may be due to the increase in mean temperatures as well as to the increase in fishing pressure (Daufresne *et al*., 2009), and latitudinal changes of climate include changes in mean temperature as well as temperature variability and seasonality (Blackburn *et al*., 1999).

In our study, we examine the temperature–body size relationships of benthic, heterotrophic ciliate protists (hereafter 'ciliates'), amoebae, nematodes, and rotifers from a network of geothermally heated small streams. The system is characterised by a natural and stable temperature gradient spanning about 15°C, while it otherwise shows little variability in its environmental features (Friberg *et al*., 2009). The nature of this system allows us to evaluate the effect of temperature on ectotherm body size by incorporating the realism of observational studies and the lack of confounding variables of laboratory-based experiments (Adams *et al*., 2013; O'Gorman *et al*., 2014).

The same study system was used by Adams *et al*. (2013) to assess the correlation between temperature and the size of diatom cells. Their study, the first to investigate the effects of temperature on diatom cells at both community and species

levels in a natural system, suggested that diatoms may represent an important exception to temperature–size rules. Diatom cell size did not show any correlation with temperature at the community level, and negative and positive correlations of cell size with temperature were almost equally represented at the species level (16 and 15 species out of 31, respectively).

Here, we provide the first assessment of temperature–body size relationships for communities of ciliates, amoebae, nematodes and rotifers in a natural system. Protists and nematodes play significant roles in the microbial food web and the cycling of organic material (Banage, 1963; Ribblett *et al*., 2005; Kathol *et al*., 2009), and rotifers provide a link between microbial and macroscopic food webs by feeding on protists and being fed on by fish and insect larvae (Arndt, 1993). Given the ecological relevance of these taxa, and that body size can affect their ecological function (Brown *et al*., 2004), knowledge of how their body size relates to temperature in natural systems is necessary in the context of ongoing climate change (Gardner *et al*., 2014).

The evidence provided for these taxa by laboratory-based studies is equivocal. The cell size of freshwater ciliates decreases by ~1.5% $°C^{-1}$ compared to their average size at 15 °C, whereas the cell size of freshwater amoebae is not significantly affected by temperature changes (Atkinson, 1994). The body size of both nematodes and rotifers is negatively correlated with temperature, but this evidence is from only one species for each taxon (nematode *Caenorhabditis elegans* and rotifer *Synchaeta pectinata*, respectively) (van Voorhies, 1996; Stelzer, 2002).

We therefore expect:

- Natural species assemblages to confirm the trends observed in laboratory conditions, namely a negative temperature–body size relationship for ciliates, nematodes, and rotifers, and a lack of such correlation for amoebae (Expectation 1);
- The temperature–body size relationship observed at higher taxonomic levels (such as phyla) to be a reflection of similar trends at lower taxonomic levels (such as genera and classes) (Fig. 1) (Expectation 2);
- Large-bodied organisms to display a steeper temperature–size correlation than small-bodied species, in line with the hypothesis that the effects of temperature on ectotherm body size are driven by differences in oxygen availability and uptake (Expectation 3);
- Temperature effects on body size may be more or less strong depending on the

substrate on which the organisms are found. Specifically, we expect the effects of temperature on body size to be stronger in organisms found in sandy substrates than in those found on the surface of submerged rocks, because the latter are more exposed than the former to water flow, and thus to oxygen supply (Expectation 4).

We attempted at testing all these expectations at best given the data available. In some cases our analytic ability was hindered by the limited sample size, as we detail in the *Results* and *Discussion* sections below.

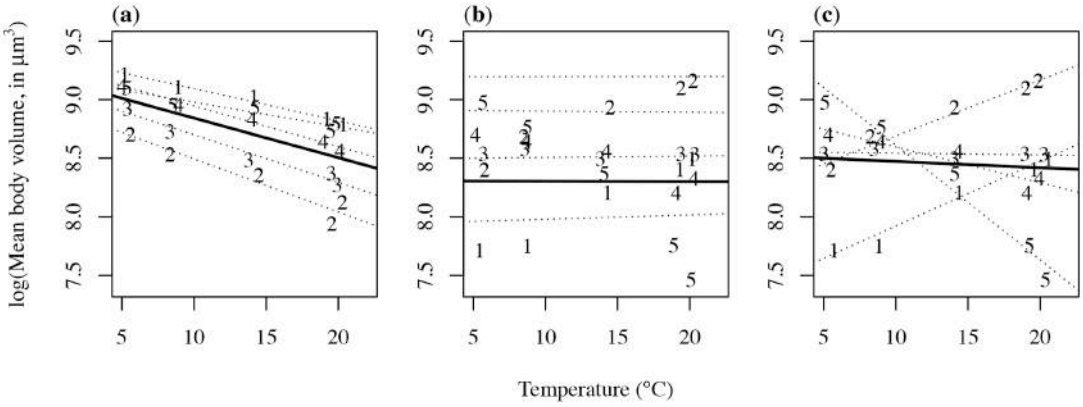

Figure 1. Alternative temperature–body size patterns at low (dotted lines) and high (solid lines) taxonomic level. Numbers 1 to 5 represent five hypothetical low taxa (such as genera or classes) belonging to the same high taxon (such as phylum). In case a negative temperature–body size relationship is widespread among low taxa, it will generate a similar trend at higher taxa (a). Alternatively, if temperature–body size relationships in low taxa are mostly neutral (b), or if negative and positive relationships are equally represented (c), they will produce an overall neutral temperature–body size relationship in higher taxa.

MATERIALS AND METHODS

Study site

Our study was conducted in six streams in a single geothermal catchment in Hengill, about 30 km east of Reykjavík, Iceland (64°.03'N: 021°.18'W; 350–420 m a.s.l.). All streams lie within ~2 km from each other. Mean stream water temperatures form a gradient from 5.4 to 20.2 °C (Tab. 1). Although there are some minor chemical

differences among the streams, these are independent of temperature, which is the major environmental gradient in the system (Woodward *et al*., 2010; Demars *et al*., 2011; O'Gorman *et al*., 2012; Adams *et al*., 2013).

Five of the six streams we studied were not manipulated experimentally, while one was the object of a warming experiment that increased the mean temperature of its lower section by 2–4 °C, compared to that of its higher section (Table 1; further details in the *Warming experiment* section below).

Table 1. Average stream temperatures recorded between 7 and 21 August 2012. Stream ID according to Woodward *et al*. (2010).

Stream ID	Temperature (°C)
10	5.4
7	5.9
11	8.6
7 (warmed section)	9–10
9	14.2
6	19.2
8	20.2

Organism sampling

Focus organisms (ciliates, amoebae, nematodes, rotifers) were sampled from each stream between 2 and 31 July 2013 in the context of an observational study and a warming experiment. This section describes the sampling techniques in common to the two studies, while differences in study design, number of replicates, and analytical approach are detailed in their specific sections below. Organisms were sampled from submerged rock surfaces in all streams, and also from coarse sandy sediment (hereafter 'sandy substratum') in the stream subjected to experimental warming. All samples were analysed within 24 hours from collection. Organisms from submerged rock surfaces were sampled as follows. Each rock was haphazardly selected and removed from its stream, and an area of 36 × 24 mm from the upper surface of each

rock was brushed with a clean toothbrush, using a 35 mm film slide frame as a sampler. Brushed material was washed off the toothbrush into a 50-ml sterile plastic tube using a squirt bottle filled with natal stream water, until the tube contained 50 ml. Sandy substratum samples were collected following the methodology described by Plebani *et al*. (2015).

In the laboratory, the contents of each vial were homogenised by gently shaking the tubes, and 1 ml of water was withdrawn (following O'Gorman *et al*. 2012). The withdrawn volume was poured into a Sedgewick-Rafter cell counter and searched using either a Nikon Eclipse E200 compound microscope or an Olympus OLY28-10-CB inverted microscope connected to a Nikon D800 reflex camera. Both microscopes were used at 100–400× magnification. Ciliates were identified *in vivo* to the lowest possible taxonomic level, termed operational taxonomic units (hereafter OTUs), using identification keys (Foissner *et al*., 1991; Foissner & Berger, 1996; Patterson & Hedley, 2009); in most cases, an OTU corresponds to a genus. Amoebae were recorded on a morphological basis as 'naked' or 'shelled'. We recorded amoebae from both groups, but only shelled amoebae (hereafter simply 'amoebae') were measured and used in this study. We classified nematodes and rotifers only to the level of phylum.

We measured the length and width of each organism *in vivo*, apart from nematodes, for which only length was recorded. Length and width measurements of organisms observed on the Nikon Eclipse E200 compound microscope were recorded to the nearest ten micrometres using ocular micrometres, while length and width measurements of organisms photographed using the OLY28-10-CB inverted microscope were recorded to the nearest micrometre using the image analysis software ImageJ. The length and width of each individual was used to estimate the corresponding volume by approximating the shape of each organism to standard geometric forms (Ruttner-Kolisko, 1977; Hillebrand *et al*., 1999).

Temperature data

Stream temperatures from the sampling period (June 2013) were not available, therefore we relied on mean stream temperatures recorded between 7 and 21 August 2012 as reported by Plebani *et al*. (2015) (Table 1). This compromise is acceptable because, although stream temperatures can show some intra- and inter-annual

variability, these changes do not affect the relative position of the streams within the temperature gradient (Fig. 2).

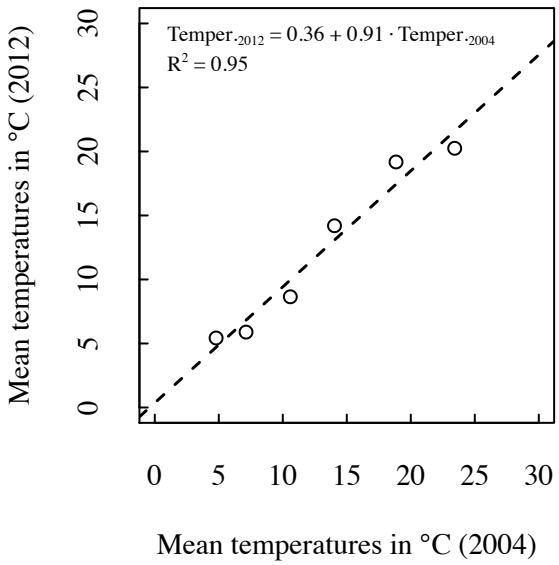

Figure 2. Comparison of stream temperatures measured in 2012 and in 2004.

Observational study

The effect of temperature on the size of ciliates, amoebae, nematodes and rotifers was evaluated via an observational study based on five unmanipulated streams. Between 2 and 6 July 2013, five rocks were chosen from each stream, marked, and sampled as described above in section '*Organism sampling*'. Expectation 1, namely a negative correlation between temperature and organism volume for each of the four organism groups under study (ciliates, amoebae, nematodes and rotifers, respectively), was assessed by fitting a mixed effect linear model for each group, with individual volumes (lengths in the case of nematodes) as response variable and environmental temperature (°C) and measurement method (three categories: 'measured by MP using ocular micrometres', 'measured by KEF using ocular micrometres', or 'measured from photographs') as the explanatory variable. Volumes were natural-log-transformed to normalize their probability distribution. We specified replicates nested in stream identity as random effects affecting the intercept of the response variable. Furthermore, for ciliates, we investigated whether the temperature–body size relationship observed at higher taxonomic levels is a reflection of similar trends at lower taxonomic levels (Expectation 2) by running the mixed effect linear model

described above for taxa lower than phylum. We used only those taxa that were present in at least three streams with at least ten individuals in each stream, which restricted this analysis to classes Holothricha, Hypothricha, and Pseudomicrothoracida. Finally we assessed whether large-bodied organism groups display a steeper negative temperature–size correlation compared to small-bodied groups (Expectation 3) by graphically comparing the sign and magnitude of the temperature–size correlation of ciliates and amoebae versus nematodes and rotifers.

All mixed effect models were implemented using function lmer() of the 'lmerTest' package version 2.0-25 (Bates et al., 2014; Kuznetsova et al., 2015) in R version 3.2.0 (R Core Team, 2015).

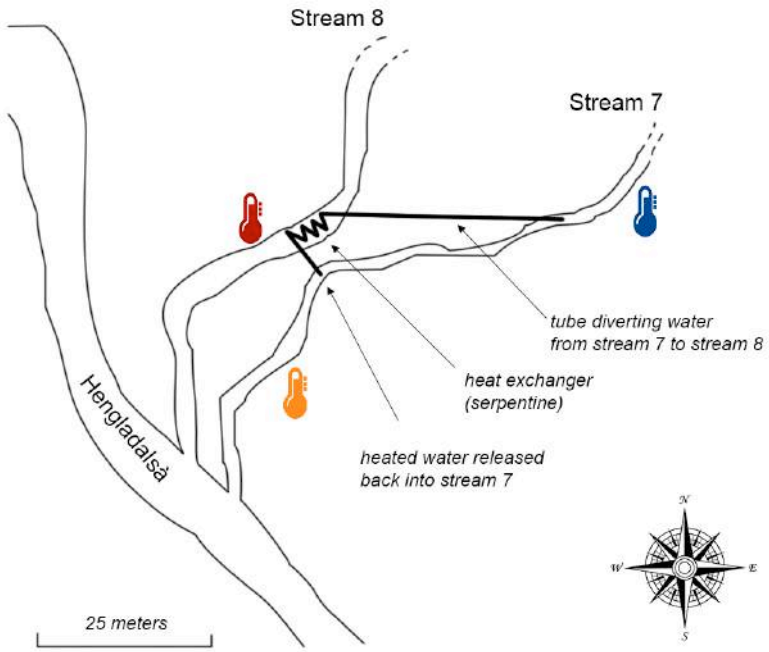

Figure 3. Diagram of the warming experiment. Temperatures of stream 8 and stream 7 (warmed and unwarmed) are reported in Table 1.

Warming experiment

One of Hengill streams, referred to as Stream 7 by Woodward *et al*. (2010), was the object of a warming experiment from 22 October 2011 until October 2013. The stream is ~15 m long, ~1 m wide, and has a natural mean temperature of 5.9 °C. Parallel to it, at about seven meters distance, lies a stream similar in size but with a mean temperature of 20.2 °C (Stream 8 in Woodward *et al*., 2010). A heat exchanger

was set up using the warm stream as a heat donor. Water from Stream 7 was diverted to the heat exchanger, warmed, and channeled back, without causing any exchange or mixing of water. This procedure increased mean temperature by 3–4 °C in the downstream half of Stream 7 (Fig. 3).

Between 9 and 31 July 2013 we sampled ciliates, amoebae, nematodes, and rotifers from submerged rock surfaces and sandy substratum in the warmed and unwarmed sections of Stream 7 (see section '*Organism sampling*' for details). We collected six rock samples and seven samples of sandy substratum from the warmed section of the stream, and six rock samples and eight samples of sandy substratum from the unwarmed section.

We investigated the effect of experimental warming on the body size of ciliates, amoebae, nematodes, and rotifers (Expectation 1), and whether any effect depends on the substrate type (Expectation 4), by fitting a factorial mixed effect model to each group separately, defining individual volume as the response variable and temperature ('natural' and 'warmed'), substrate type ('rock' and 'sand'), and measurement method ('measured by MP using ocular micrometres', 'measured by KEF using ocular micrometres', or 'measured from photographs') as categorical explanatory variables. Organism volumes were natural-log-transformed to normalize their probability distribution. The random effect was specified as detailed in section '*Observational study*', above. Finally we evaluated whether multicellular, large-bodied organism groups (nematodes and rotifers) display a steeper temperature–size correlation compared to unicellular, small-bodied groups (ciliates and amoebae).

All mixed effect models were implemented using function lmer() of the 'lmerTest' package version 2.0-25 (Bates et al., 2014; Kuznetsova et al., 2015) in R version 3.2.0 (R Core Team, 2015).

RESULTS

Observational study

In total we identified and measured 634 ciliates from 19 OTUs and 13 classes, 107 amoebae, 34 nematodes, and 129 rotifers. Temperature explained only between 0.01% and 8.91% of the observed variability in body size (Table 2). In contrast with

Expectation 1, neither ciliates, amoebae, nematodes, nor rotifers displayed significant overall temperature–body size correlations (Fig. 4a-d; Table 2).

For ciliate taxa lower than phylum, body size was significantly, negatively correlated with temperature only in Pseudomicrothoracida (Fig. 6a-c; Table 3), suggesting that the lack of a temperature–body size relationship observed at higher taxonomic levels is a reflection of lack of consistency in the relationship across lower taxonomic levels (Expectation 2).

Contrary to Expectation 3, large-bodied organisms did not display a steeper temperature–size correlation compared to small-bodied species. The correlation between temperature and body size was equally weak in generally large-bodied groups (nematodes and rotifers) and in generally small-bodied groups (ciliates and amoebae) (Fig. 5, Table 2).

Warming experiment

In total we identified and measured 699 ciliates from 31 OTUs and 13 classes, 125 amoebae, 73 nematodes, and 170 rotifers. Neither ciliates, nematodes, nor rotifers displayed a significant overall temperature–body size correlation (Fig. 7a, 7c, 7d; Table 4), thus Expectation 1 was not supported. Amoebae found on sandy substrates showed a significant reduction in size by ~60% in the warmed section compared to the unwarmed (SE = 26.6%, t = -2.2778, p = 0.0250), regardless of the measurement method (Fig. 7b; Table 4). This contrasts with results from laboratory studies, but is in line with the general expectation of ectotherms becoming smaller with increasing temperature.

A comparison of the effect of temperature on body size in organisms from rocky or sandy substrates was possible only for ciliates and rotifers. In contrast to Expectation 4, the mean body size of both groups did not differ significantly in the warmed and unwarmed section of the stream, regardless of the substrate type from which the organisms were sampled (Fig. 7; Table 4).

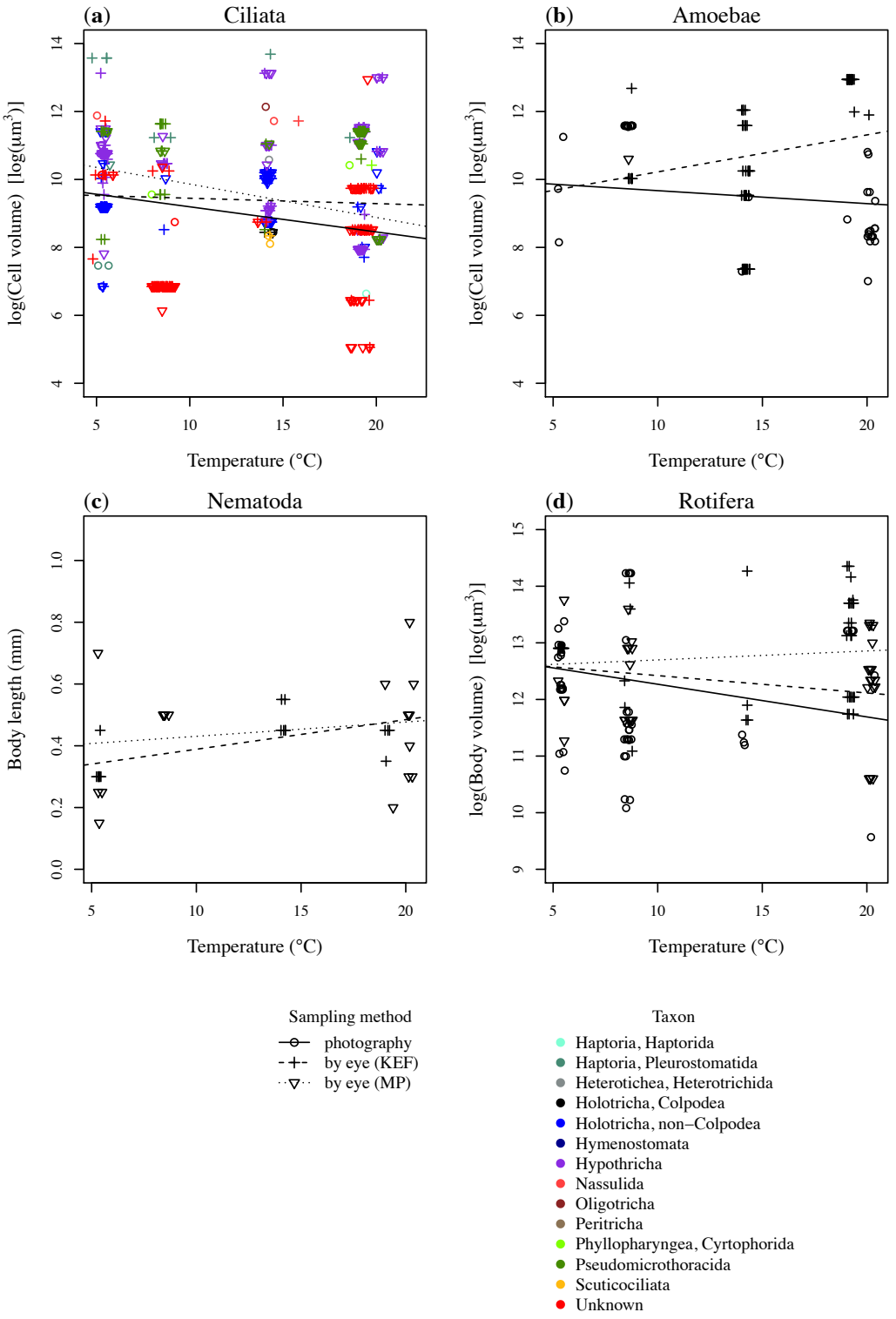

Figure 4. Relationship between the size of ciliates (a), amoebae (b), nematodes (c), and rotifers (d) and stream temperature. Volume estimates are natural-log-transformed. Artificial variability in stream temperatures was added to improve data visualization. None of the regression lines represent a significant trend. Details in Table 2.

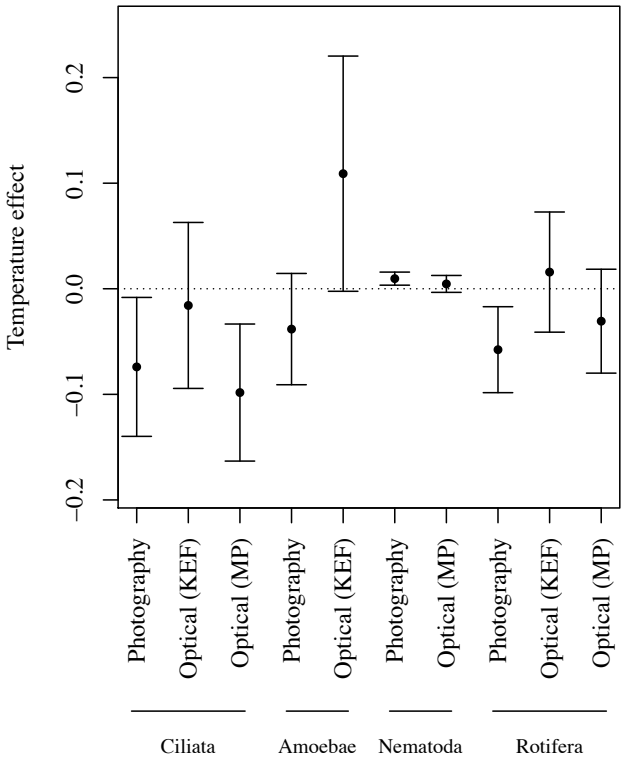

Figure 5. Temperature effects measured in the observational experiment. Details in Table 2.

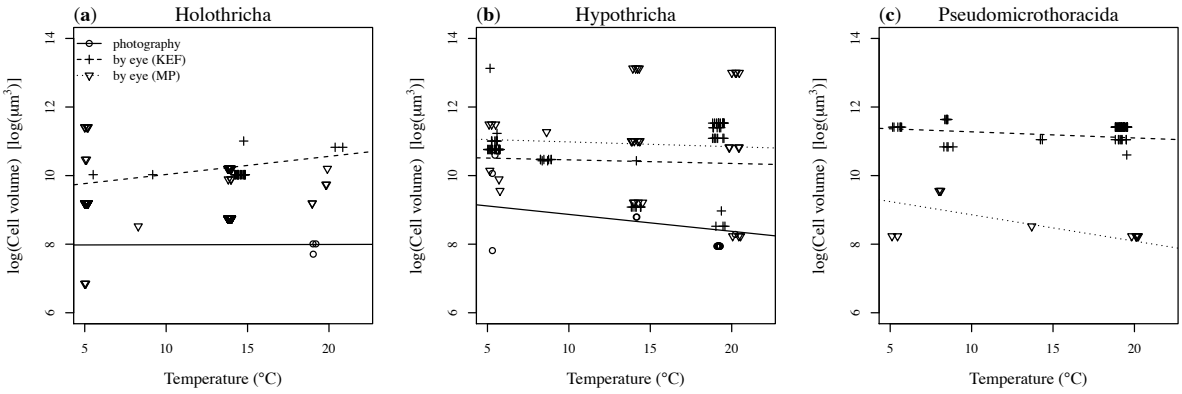

Figure 6. Relationship between cell size and stream temperature for ciliate groups Holothricha (a), Hypothricha (b), and Pseudomicrothoracida (c). Volume estimates are natural-log-transformed. Artificial variability in stream temperatures was added to improve data visualization. Details in Table 2.

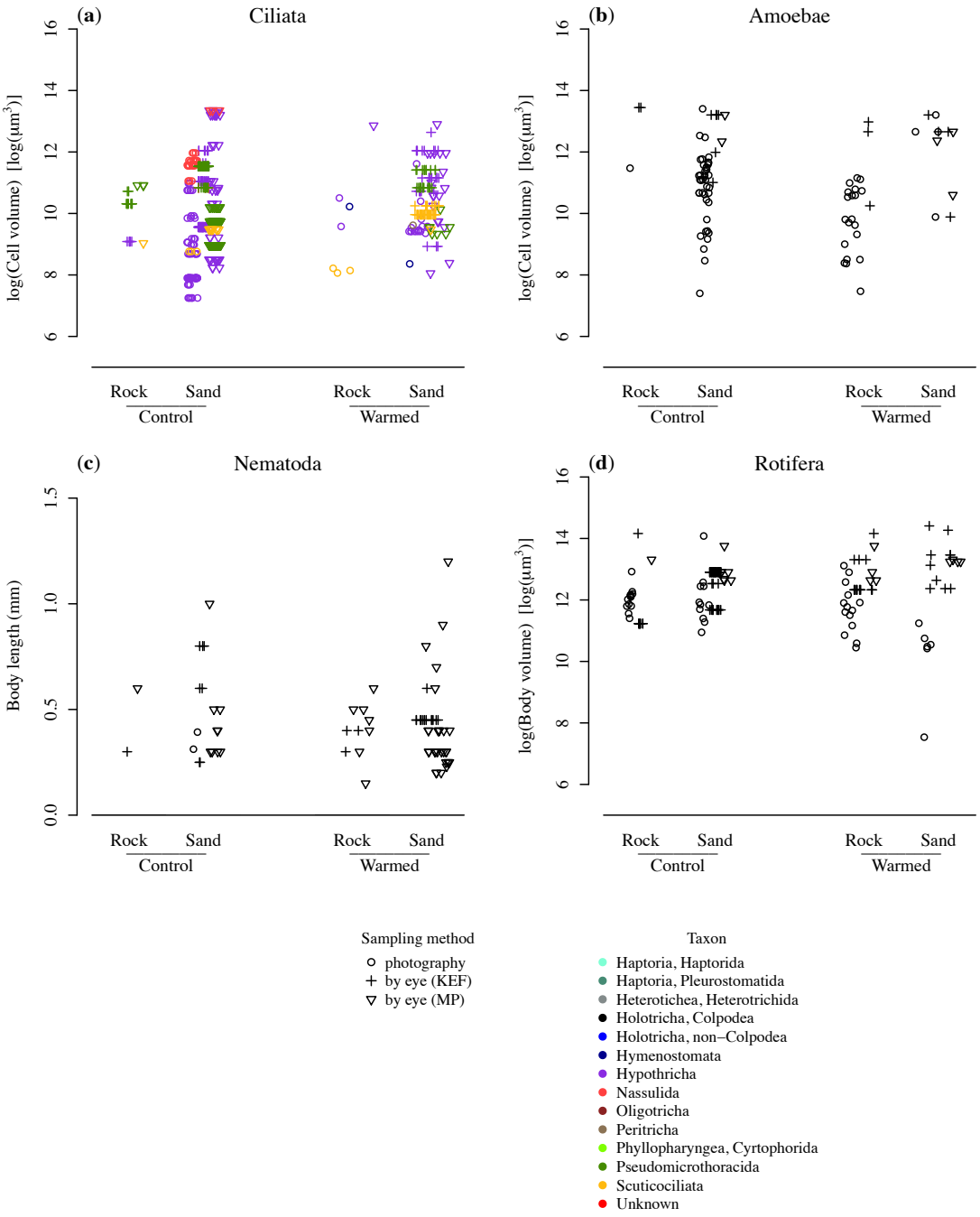

Figure 7. Individual size of ciliates (a), amoebae (b), nematodes (c), and rotifers (d) under different warming conditions and on different substrates. Volumes are natural-log-transformed. Data points were jittered on the x-axis to facilitate visualization. Details in Table 4.

Table 2. Results of the mixed-effect models assessing the correlation between stream temperature and organism size for ciliates, amoebae, nematodes, and rotifers.

Group	Sampling method	Temperature effect	SE	df	t value	p value
Ciliata	Photography	-0.0740	0.0658	42.6575	-1.1235	0.2675
	Optical (KEF)	-0.0158	0.0786	29.3853	0.7409	0.4646
	Optical (MP)	-0.0983	0.0649	265.8908	-0.3737	0.7089
Amoebae	Photography	-0.0382	0.0527	3.2813	-0.7248	0.5169
	Optical (KEF)	0.1090	0.1114	12.8419	1.3212	0.2095
Nematoda	Photography	0.0096	0.0062	30.0000	1.5355	0.1351
	Optical (MP)	0.0046	0.0080	30.0000	-0.6169	0.5419
Rotifera	Photography	-0.0577	0.0407	12.5685	-1.4169	0.1808
	Optical (KEF)	0.0158	0.0569	6.4981	1.2930	0.2401
	Optical (MP)	-0.0307	0.0492	33.1576	0.5496	0.5863

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Table 3. Results of the mixed-effect models assessing the correlation between stream temperature and organism size for ciliate groups Holothricha, Hypothricha, and Pseudomicrothoracida.

Group	Sampling method	Temperature effect	SE	df	t value	p value
Holothricha	Photography	0.0009	0.0320	3.9648	0.0293	0.9781
	Optical (KEF)	0.0529	0.0665	10.0288	0.7814	0.4526
Hypothricha	Photography	-0.0493	0.0630	14.4809	-0.7836	0.4459
	Optical (KEF)	-0.0105	0.0685	19.2603	0.5672	0.5772
	Optical (MP)	-0.0139	0.0689	50.4592	0.5139	0.6096
Pseudomicrothoracida	Optical (KEF)	-0.0179	0.0048	74.8215	-3.7135	0.0004 ***
	Optical (MP)	-0.0769	0.0114	76.6132	-5.1825	<0.0001 ***

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

Table 4. Results of the mixed-effect models assessing the effect of warming, substrate type, and their interaction on the size of ciliates, amoebae, nematodes, and rotifers. Too few amoebae and nematodes were measured from rock surfaces in the unwarmed section of the stream. In this case, only the contribution of warming to the variability in size observed from sandy samples was measured.

Group	Substrate type	Sampler method	Temperature effect	SE	df	t value	p value
Ciliata	Rock	Photography	-0.3133	1.0448	83.4383	-0.2999	0.7650
		Optical (KEF)	-3.2330	1.9248	675.0388	-1.5168	0.1298
		Optical (MP)	1.0919	1.2874	170.9963	1.0915	0.2766
	Sediment	Photography	-1.1753	1.2139	53.9649	-0.7101	0.4807
		Optical (KEF)	4.4012	2.0031	650.5609	1.6853	0.0924
		Optical (MP)	-0.7349	1.4676	143.4820	0.5888	0.5569
Amoebae	Sediment	Photography	-0.6059	0.2660	93.0000	-2.2778	0.0250 *
		Optical (KEF)	0.0715	0.8305	93.0000	0.0861	0.9316
		Optical (MP)	-0.2906	1.1439	93.0000	-0.2541	0.8000
Nematoda	Sediment	Photography	-54.1659	79.6709	11.4896	-0.6799	0.5101
		Optical (KEF)	-69.3158	128.5844	5.7819	-0.5391	0.6100
Rotifera	Rock	Photography	-0.2426	0.6299	7.8171	-0.3852	0.7104
		Optical (KEF)	0.4004	1.0036	6.7860	0.6407	0.5427
		Optical (MP)	-0.2549	1.0242	18.2132	-0.0120	0.9906
	Sediment	Photography	-1.6455	0.8317	9.0373	-1.6868	0.1258
		Optical (KEF)	1.7368	1.3642	6.8040	1.1455	0.2907
		Optical (MP)	1.2280	1.1868	25.0197	1.7094	0.0998

*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$.

DISCUSSION

Evidence from most laboratory-based experiments suggests that ectotherms follow an inverse temperature–body size relationship, similar to Bergmann's Rule in endotherms, but driven by different mechanisms. Yet, results from field studies produce a more heterogeneous and taxon-dependent picture. In an attempt to clarify this contradiction, we studied the effect of temperature on the mean body size of ciliates, amoebae, nematodes, and rotifers from a geothermal stream network. In our system we found a widespread lack of correlation between temperature and body size.

In contrast to our main expectation, our observational study did not reveal any significant correlations between the size of the study organisms and temperature. Moreover, nematodes and rotifers did not appear to display a steeper negative correlation of their body size with temperature than ciliates and amoebae did, in contrast to the expectation that the strength of the response of body size to temperature would depend on the average size of the study groups. Our findings conflict with the significant, negative correlations between temperature and the size of ciliates, nematodes, and rotifers observed in controlled laboratory conditions (Atkinson *et al*., 2003; van Voorhies, 1996; Stelzer, 2002), suggesting that temperature-body size patterns observed in controlled environments cannot be directly used to make predictions for natural systems.

The results from our field-based warming experiment agree for the most part with those of the observational study, with the notable exception of amoebae. While amoebae body size was not correlated with temperature in our observational study, it showed a significant reduction with increased temperature in our warming experiment. However, this was only the case for amoebae from sandy substrates. This is in line with our expectation that sandy substrates are more oxygen-limited than rock surfaces, and thus harbor organisms that respond more strongly to temperature effects mediated by the limiting oxygen. This result brings partial support to the hypothesis that the negative temperature–body size correlation in ectotherms is mediated by oxygen limitation (Forster *et al*., 2012; Horne *et al*., 2015). We did not measure water oxygen content for the two substrate types, and thus further studies are required to confirm the role of oxygen availability in producing temperature–body size relationships. Regardless of whether the reduction of amoebae body size with temperature is linked to oxygen availability or not, the fact that only one of the four organism groups we studied exhibited temperature–body size relationships undermines the universality of negative temperature–body size relationships, as well as of oxygen availability as a general underlying mechanism for such relationships.

A previous study undertaken in the same geothermal stream network showed that the size of diatoms is unrelated to temperature at the level of community, and that negative and positive temperature–body size correlations are about equally represented at the level of species, suggesting that diatoms may represent an exception to the general rule (Adams *et al*., 2013). Our study suggests that diatoms may not be an exception among aquatic micro-invertebrates. The temperature–size

rule might fail to apply to natural communities more often than not, even in those systems in which temperature arguably represents the most important gradient.

The lack of a generalized, negative correlation between body size and temperature in natural communities can have several explanations. One possibility is that processes occurring in natural systems can obscure the temperature effects observed on the body size of organisms reared in controlled conditions. For example, increases in the mean intensity or variance of an environmental process can lead to opposite ecological effects (e.g. Benedetti-Cecchi *et al*., 2006). Simulations have demonstrated that temperature variability and mean temperature can have positive or negative effects on a species' generic 'performance', depending on the shape of the species' temperature performance curve (Vasseur *et al*., 2014). Natural temperature variability may be sufficient to counteract the effects of mean temperature, for example because temperature changes occur more rapidly than the time it takes for the size of organisms to respond. In our system, streams whose temperature is farther from the average local environmental temperature are more prone to large temperature fluctuations (e.g. when it rains, the temperature will drop further away from the mean in warm streams than in cold streams). This may be sufficient to mask the effects of mean temperature on ectotherm body size in our study system, and perhaps in natural communities in general.

Alternatively, a negative temperature–size correlation may not be universal as it has been suggested, but instead it may depend on the temperature niche of species. This has been observed in North American freshwater fish, where warm-water species tend to decrease in size with decreasing temperature, while cold-water species show the opposite trend (Rypel, 2014).

These possibilities require further evaluation with both laboratory-based and field-based experiments on a larger number of species from different higher taxa.

Our findings are of particular relevance in the context of ongoing global climate change. Declining body size may not be, as it has been suggested, a universal response to warming (Gardner *et al*., 2011). Thus, models predicting the ecological effects of environmental temperature on species fitness should be developed accordingly, by accounting for the idiosyncratic effects of environmental temperature on body size in an empirically defined, taxon-specific way, instead of using a 'one-size-fits-all' approach.

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6

General Discussion

GENERAL DISCUSSION

The work presented in this thesis studied the ecological effect of temperature on the population dynamics, diversity, and body size of freshwater ciliate protists. By using microcosm- and natural experiments we were able to examine the interactive effects of temperature and habitat size, substratum type, species identity, interspecific interactions, and thermal adaptation. These studies also allowed testing the validity of some of the predictions of the Metabolic Theory of Ecology (MTE).

All the variables we tested, both abiotic (habitat size, substratum type) and biotic (species identity, thermal adaptation, occurrence of interspecific interactions) produced two-way or three-way interactions with temperature for some of the response variables. Here I will discuss our main results, their implications, and possible lines for future research.

In *Chapter Two* we studied possible interactive effects of changes in temperature, habitat size, and interspecific competition on population dynamics. This study is the first to address the joint ecological effects of temperature and habitat size experimentally. All experimental variables affected population growth, maximum density, and the rate of population decline. We found that temperature can have a stronger negative effect on the rate of population decline of some species when they are reared in small habitats, and in the presence of a competing species. This interactive effect contrasts with our expectations, and how it arises is unclear. We expected that population dynamics parameters would have scaled with temperature in response to its metabolic effects, while we expected habitat size to affect only maximum population density in a linear fashion via its effect on resource availability. We suggest that the interactive, community-mediated effects of temperature and habitat size on population decline rate may be due to edge effects. Edge effects can affect predator-prey interactions (Bergström and Englund 2004), thus we hypothesize that they may also alter the rate at which species compete in a temperature-dependent fashion. Future studies may test this hypothesis by comparing the demographic effects of temperature and competition in habitats of the same size, but differing in their volume:depth ratio, or in their ratio of volume to submerged surface area. Our findings also have relevant implications for conservation biology, suggesting that the

negative effects of climate warming on population dynamics can be limited by preserving natural habitats.

In *Chapter Three* we demonstrated that thermal adaptation could affect the way the population dynamics of some species respond to temperature, as well as the temperature-dependence of interspecific interactions. Thus, models that do not account for thermal adaptation (e.g. Deutsch et al. 2008) may predict worse-than-likely scenarios regarding the ecological consequences of climate change. Yet, accounting for thermal adaptation in ecological models is complicated by the fact that the degree of thermal adaptation varies, depending on the population parameter, on the species identity, and on the temperature to which organisms are exposed. Future studies should delve deeper into the mechanisms underlying these differences. Some species may have larger adaptive potentials to the environmental temperature than others because their genomes are subject to different mutation rates. Also, thermal adaptation may occur at the individual level but not at the population level. For example, organisms may adapt by optimizing swimming speed and body size (Angilletta 2009), while their population dynamics may remain unaffected. To unveil the trade-offs in the adaptive potential of different traits and different species, future studies should adopt an integrated approach, linking genetics, physiology, and ecology.

The results we presented in *Chapter Two* and *Chapter Three* bring partial support to the predictions of the MTE. In the temperature range in which intrinsic growth rate was positively correlated with environmental temperature, an exponential, metabolism-based model was always at least as good as a linear regression model in describing the data. Yet, observed parameter values of the exponential correlation between temperature and growth rate often diverged from the predicted, universal mean value of 0.6–0.7 eV (Brown et al. 2004). Differences were due to species identity and to thermal adaptation. This suggests that species identity and thermal adaptation may affect the activation energy of metabolic reactions, or the strength of the correlation between metabolic and ecological rates. Further studies should address this possibility, for example by measuring the correlation of organisms' respiration with their population growth rate.

In *Chapter Four* we described a substratum-dependent effect of temperature on the diversity and total biomass of natural assemblages of freshwater ciliates. We suggest that the negative effect of temperature on the diversity and total biomass of

ciliate assemblages from rock surfaces may be mediated by the feeding activity of invertebrate grazers. This hypothesis can be tested by experimentally excluding grazers from some rock surfaces, and then comparing the diversity and abundance of their ciliate assemblages to those from rocks accessible to grazers. Moreover, future studies in our study system could also increase the taxonomic resolution by using a metagenomic approach.

In *Chapter Five* we tested the generality of the negative correlation between temperature and the body size of ectotherm assemblages in a natural system. Laboratory experiments generally supports this negative temperature–body size correlation. On the contrary, only one family of ciliate protists and amoebae found in sandy substrata displayed a negative correlation of their body size with temperature. We suggest that the temporal variability of temperature in nature may be sufficiently large to obscure the expected correlation of body size with temperature. Alternatively, the general lack of temperature–size correlation we observed at a low taxonomic resolution may reflect a mix of positive and negative correlations between temperature and body size at the species level. This, in turn, may be a reflection of a species' thermal niche (Rypel 2014).

To conclude, in my thesis I could show, together with my collaborators, that temperature has widespread ecological effects. My findings bring partial support to the theory according to which ecological effects of temperature are largely generated by the temperature-dependence of metabolic rates. Yet, both biotic and abiotic variables can modulate the ecological effects of temperature. Moreover, ecological patterns observed in controlled conditions can be obscured in natural environments, possibly by the natural variability of environmental temperature, by a species' thermal niche, or by unpredicted trophic interactions. The research presented in my thesis supports the efficacy of both microcosm experiments and natural experiments for testing ecological theories and evaluating their robustness in the face of the variability and complexity of natural systems.

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